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American Journal of Veterinary Research

Volume V JULY, 1944 Number 16

Studies on Brucellosis of Swine: I. Infection Experiments with Weanling Pigs

L. M. HUTCHINGS, B.S., D.V.M., M.S., A. L. DELEZ, D.V.M., M.S., and C. R. DONHAM, D.V.M., M.S.

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RELATIVELY few controlled experiments have been conducted with brucellosis of swine. Nearly all of our few supposed facts concerning this disease need to be reëxamined. The literature concerning brucellosis of swine is contradictory especially in regard to the mode and duration of infection, the age incidence, and agglutination response. More accurate information concerning the mode of infection in swine of various ages is essential before further studies such as period of incubation, duration of infection, symptoms, vaccination procedures, or control measures can be studied in an adequate manner. Until it is known how to consistently infect swine, we cannot measure accurately the results of studies on other phases of the problem.

Connaway, Durant, and Newman[1] have produced infection by way of feeding and also infection was transmitted from reacting sows to two boars by copulation. They believed pregnancy was not necessary to establish infection. Suckling pigs from reacting sows showed a positive reaction at birth, or more often, soon after. They found this reaction to persist in the pigs for three to fifteen weeks. The possibility of extrauterine infection was not excluded. The passive transfer of agglutinins from colostrum to the pigs may have caused the early agglutination reactions. These authors believed that suckling pigs from reacting sows may be raised free from brucellosis if isolated after weaning.

Thomsen[2] showed that in Denmark suckling pigs carried infection, acquired by feeding *Brucella suis*, as long as one year after ingestion. He found gilts may be infected by copulating with infected boars, but not regularly. He infected boars *per os* and postulated that the conjunctiva might be a portal of infection.

Hadley and Beach[3] fed *Br. suis* to 4 gilts from one week to one month prior to breedings. All reacted to the agglutination test but only 1 of the 4 aborted. The authors stated, "Since only 1 of these 4 actually aborted, it is evident that normally gilts exhibit considerable resistance to, or have an appreciable amount of immunity against, porcine abortion bacilli when introduced *per os* before conception has occurred." They exposed a nonreacting boar to an intrapreputial injection of *Br. suis* after which he bred 3 nonreacting sows at intervals. One sow aborted; the other 2 farrowed normally. The boar showed a positive reaction to the agglutination test for a period of ninety days.

Graham, Boughton, and Tunnicliff[4] reported the results of feeding 22 male pigs, 1 1/2 months old, one dose of porcine Brucella culture with only weak agglutination response. They concluded, "As judged by the agglutination test . . . young pigs are quite resistant."

Howarth and Hayes[5] reported that of 43 young pigs from 8 reacting sows, which

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TABLE 1—Results of Exposing Weanling Pigs to Brucella Suis per Rectum
(Date of exposure June 15, 1942)

Pig Sex Litter	Pre-Exposure Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after exposure) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Bacteriologic Results at Autopsy	Farrowing Results[2]
904	-----	++PT-	PTT--	PT---												Positive	
Boar						Destroyed 8-14-42										60 days	
L 1		Neg.	Pos.	Neg.													
930	-----	TT---	TT---	TT---	T----	+PT--	TT---	-----	-----	++T--						Negative	
Boar											Destroyed 12-3-42					171 days	
L 5		Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.							
936	-----	T----	T----	+PT--	+PT--	+++T-	++PT-	++PT-	+++T-	++PTT	+PT--	P----	P----				
Boar														Sold			
L 6		Neg.	Pos.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.				
949	-----	+PT--	+P---	+PT--	++P--	+++PT	++PT-	++PT-	++P--	++T--	+P---	+----	PT---				
Boar														Sold			
L 8		Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.				
975	-----	+PT--	T----	TT---	-----	T----	TT---	T----	-----	-----							
Barrow											Sold						
L unknown		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.							
908	-----	+PTT-	++PT-	++PT-	+++P-	+++T-	+++PT	++P--	+PT--	+PT--						Negative	
Barrow											Destroyed 11-24-42					162 days	
L 1		Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.							
920	-----	T----	TT---	T----	T----	TT---	TT---	T----	-----	TT---	+PT--	P----	+P---	T----	+PT--		
Female																	Normal
L 3		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
923	-----	T----	-----	-----	T----	+PT--	+P---	+P+P-	++++-	++PT-	+++-	P----	T----	PTT--	+PT--		Not
Female																	Pregnant
L 4		Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.			Neg.	Neg.	Neg.		
954	-----	+PT--	+PT--	+PT--												Positive	
Female					Destroyed 8-14-42											60 days	
L 8		Pos.	Pos.	Neg.													
972	-----	+P---	++P--	++PT-	++P--	+PT--	T----	T----	-----	-----[3]	-----	-----	-----	-----	PT---		
Female																	Normal
L unknown		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		

(196)

1 +=Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

2 Normal, indicates the litter was normal and the cultures from fetal membranes, milk, and destroyed or dead pigs were negative for Br. suis to both direct cultures and guinea-pig inoculation methods.

3 Intradermal injections of Brucella extracts applied prior to this test.

had been penned together with these and other reacting swine, only 9 showed any degree of agglutination. They concluded, "One is, therefore, led to believe that young swine do not become infected readily up to 4 months of age." This seems contrary to the findings of Thomsen. McNutt[6] stated that infection may result from most all modes of entrance.

Conflicting views are held in regard to the interpretation of the agglutination test as a diagnostic procedure for brucellosis in swine.

Connaway, Durant, and Newman[1] used both the agglutination method and the complement fixation test. They concluded the latter was preferable because of its greater accuracy.

Hadley and Beach[3] concluded that agglutination tests indicate that the abortion organisms do not produce disease in young pigs.

Thomsen[2] states, "Agglutination in 1:100 (and higher dilutions) signifies that the animal is—or has been infected. Agglutination of 1:50 is at any rate to be considered very suspicious, and it calls for further examination which, as a rule, will show that the animal is infected. . . . Often the antibody production takes place but slowly so that aborting sows not infrequently show no serum reaction until after the abortion. Often the titer values of the infected animals are rather low. It looks as if the reaction power of an infected animal gradually subsides more or less completely in spite of persistent infection."

Fitch[7] did not consider a reaction in a 1:25 dilution as indicating infection but believed that even a partial reaction in a 1:50 dilution should be regarded as suspicious and that a reaction in a 1:100 dilution should be considered positive evidence of past or present infection.

Carpenter[8] did not consider that the agglutination of porcine serum in a 1:25 dilution was a specific reaction. Johnson and Huddleson[9] reported the rapid agglutination test was an accurate method of following the course of Brucella infection in swine. They considered all reactors, even though reacting to a slight degree, usually have some significance.

McNutt[10] stated, "Of 12 reacting (swine) at 1:50, 50 per cent yielded Brucella cultures, while of 15 reacting above 1:50, a little less than 50 per cent yielded positive cultures; about the same in either case." He also stated that a reaction of 1:25 appears to be suspicious. Of 5 such animals cultured, none yielded cultures. With this in mind, one might class 1:25 reactors with the uninfected. Later McNutt[6] reported that a reaction in a dilution of 1:25 constitutes a positive reaction and indicates infection in the opinion of the majority.

Hashizume, Kamoto, and Kirizawa[11] concluded from their studies of swine brucellosis that those swine which gave a positive agglutination test with a 1:50 dilution of their serum had no lesions nor was *Br. suis* recovered, whereas those which gave a partial reaction with a 1:100 dilution of the serum did have lesions and Brucella was isolated from them. The agglutination test is suggested as a reliable method of diagnosis.

Stone[12] stated that a reaction in a dilution of 1:25 in an infected herd usually indicates infection. In a clean herd, a titer of 1:25 is more likely to represent the peak of a normal curve, but an animal with a titer of 1:25 should be considered suspicious. A titer of 1:50 or higher may safely be regarded as indicative of infection.

Cameron[13] stated: "According to the survey, a reaction at 1:25 is specific, indicating contact with Brucella, and the ultimate objective should be a herd in which all animals are negative in all dilutions. Most of the low titer reactors following artificial infection became either completely negative or positive ten weeks after infection, the numbers being approximately equal. Few remained at low titers." Later Cameron, Gregory, and Hughes[14] stated: "Most investigators assume that as a species hogs are susceptible to *Br. suis*, and the validity of the agglutination test may be questioned if an animal fails to show agglutinins after repeated experimental ex-

TABLE 2—Results of Exposing Weanling Pigs to Brucella Suis per Os
(Date of exposure June 15, 1942)

Pig Sex Litter	Pre-Exposure Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after exposure) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Bacteriologic Results at Autopsy	Farrowing Results[2]
907	-----	PT--	TT---	+PT--	T----	T----	TT---	-----	-----	-----	PT--[3]	P----	-----	T----	+----		
Female L 1		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		Normal
913	-----	+PT--	PT---	PTT--	T----	PT---	+PT--	+P---	++PT-	PT---	T----	T----	T----	T----	PP---		
Female L 2		Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		Normal
944	-----	T----	TT---	T----	-----	PT---	++PT-	++P--	+++PT	++P--	+PT--	+P---	PT---	P----	PT---		
Female L 7		Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		Normal
951	-----	+PTT-														Positive spleen kidney, mandibular and inguinal lymph nodes	
Female L 8				Died 7-1-42													
		Neg.															
967	-----	+PT--	TT---	T----	-----	-----	T----	-----	+PTT-	PT---							
Barrow L unknown		Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Sold						
968	-----	T----	T----	PTT--	PPT--	TT---	TT---	TT---	T----	T----							
Barrow L unknown		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Sold						
925	-----	-----	-----	T----	-----	-----	+PTT-[3]										
Boar L 4		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Destroyed 9-24-42								Negative	
932	-----	-----	++P--	PP---	PPT--	+P---	PT---	++P--	+PTT-	+P---							
Boar L 5		Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Destroyed 12-3-42					Negative	
938	-----	+PTT-	PT---	+PT--	+PT--	+PT--	+++PT	++++P	++++P	+++P	++T-	+++--	++T--	++---[4]	+PT--	Testes	
Boar L 6		Neg.	Pos.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.	Negative	
959	-----	+PT--	T----	+PT--	PTT--	+T---	+PTT-	+++T-	+PT--	+TT--	PT---	P----	T----			Testes	
Boar L 9		Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Castrated and sold		Negative	

[1] + = Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

[2] Normal, indicates the litter was normal and the cultures from fetal membranes, milk, and destroyed or dead pigs were negative for Br. suis to both direct culture and guinea-pig inoculation methods.

[3] Intradermal injections of Brucella extracts applied prior to this test.

[4] Castrated prior to this test.

posures. The difficulty, however, is not in the agglutination test, but to differences in genotype for resistance and susceptibility in the swine population."

Kernkamp[15] stated: "I think the agglutination test for brucellosis in swine is as reliable as is the agglutination test for this disease in the bovine. The dilution titer which I would consider as representing evidence of infection would be somewhere in the vicinity of 1:100."

Some investigators have considered brucellosis in swine to be an abortion disease, others have reported the symptom of abortion to be rare. Graham, Boughton, and Tunnicliff,[5] in discussing the results of a vaccination experiment, concluded, "It is apparent that the delivery of healthy litters by the vaccinated group may be related to time of exposure to the virus of the disease."

EXPERIMENTAL PROCEDURE

Sixty pigs were divided into 6 groups of 10 each and exposed to massive doses of a polyvalent suspension of *Br. suis* by six different routes. Litter mates were run at random in various groups.

Six different strains of *Br. suis* were used in the polyvalent inoculum. These cultures were obtained from the Michigan, Minnesota, and Iowa Agricultural Experiment stations and typed in this laboratory and found to be *Br. suis* according to the Huddleson[16, 17] dye plate method. Prior to their use for inoculum for pigs, each of the six cultures was injected subcutaneously in small doses into 2 guinea pigs to determine its virulence. Each culture produced characteristic lesions, positive agglutination reactions, and positive cultural findings in the guinea pigs.

Ten other pigs were kept in a group as controls and as a check on possible spread of the infection. These 10 were housed in a separate section of the barn and were allowed access to an exercising yard separated by a 6 ft. dead lane from the exposed swine.

The approximate inoculating doses of this polyvalent inoculum for the pigs and the routes of inoculation were as follows:

Group 1, 15 billion organisms per rectum; *group* 2, 15 billion organisms *per os; group* 3, 122 million organisms per conjunctiva; *group* 4, 3 billion organisms per vagina and prepuce; *group* 5, 9 billion organisms subcutaneously; and *group* 6, 9 billion organisms intravenously.

Following exposure, body temperatures were recorded at daily intervals for fifteen days and at intervals of approximately one week thereafter for a period of twenty weeks.

Bacteriologic examinations of the blood for *Br. suis* were conducted at intervals of approximately two weeks for five months and approximately once per month thereafter for six months. Sterile Bacto Tryptose broth in 2 per cent concentration plus 0.5 per cent salt, and 1 per cent sodium citrate was employed as the blood culture medium. The medium was placed in 20 cc. amounts in bottles, with skirt-type rubber stoppers, suitable for puncturing with a hypodermic needle. This medium was inoculated with 2 to 5 cc. of blood immediately after collection and shaken to prevent clotting of the blood. Following incubation at 37 C. for eight days, 1 cc. amounts of the blood broth culture were plated on the surface of Tryptose agar plates and reincubated.

Serum agglutination tests were conducted at intervals corresponding to those of the blood cultures. Standardized antigens, as supplied by the United States Bureau of Animal Industry, were employed for such tests using both the test tube and rapid methods. Rapid agglutination tests were conducted in dilutions commonly used comparable to the dilutions (1:25, 1:50, 1:100, 1:200, and 1:400) of the test tube method.[18] Test tube agglutination tests were conducted in dilutions of 1:10, 1:25, 1:50, 1:100, and 1:200. Although some variations in the results on the same sample occurred between the two methods of testing, in general, the agreement was good.

Bacteriologic examinations were made to determine the localization of *Br. suis* in the various tissues of the bodies of some of the artificially exposed pigs. Selected pigs from the experimental groups with varying maximum agglutination titers were destroyed at appropriate intervals for such examinations. The following tissues were examined: liver, spleen, lungs, kidneys, regional lymph nodes, testis, uterus, and ovary.

Both the direct culture and guinea pig inoculation methods were used. Direct cultures were made, using aseptic technique, and Tryptose agar plates. The guinea pigs inoculated with tissues from these pigs were destroyed and bacteriologic examinations made after approximately four week periods of incubation. The same procedures were followed in culturing semen, fetal membranes, dead and destroyed baby pigs, colostrum, and milk.

Tissues were collected for histopathologic study from all swine that were destroyed or that died in the course of the experiment.

At the conclusion of approximately six months, the pigs which remained were grouped according to sex regardless of the route of exposure. A total of 26 gilts remained of which 3 were from the control group and not exposed and 23 had received the various exposures pre-

TABLE 3—Results of Exposing Weanling Pigs to Brucella Suis per Conjunctiva
(Date of exposure June 15, 1942)

Pig Sex Litter	Pre-Exposure Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after exposure) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Bacteriologic Results at Autopsy	Farrowing Results[2]
909	-----	PT---	+PT--	PT---	T----	T----	T----	-----	-----	-----	++PT-[3]	P----	-----	T----	Not done		Normal
Female L unknown		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.			
926	-----	TT---	-----	T----	-----	-----	PTT--	+PT--	++PT-	+PT--	+PT--	PP---	-----	PT---	PT---		Normal
Female L 4		Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
935	-----	T----	-----	TT---	-----	-----	T----	T----	-----	-----	++PT-[3]	T----	P----	T----	PT---		Normal
Female L 6		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
948	-----	PT---	-----	T----	T----	+P---	+PTT-	++P--	++PT-	++P--	++PT-	++---	PT---	T----	P----		Normal
Female L 8		Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
963	-----	++PT-	TT---	T----	-----	-----	++PTT[3]										
Barrow L unknown		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Destroyed 9-24-42								Negative	
901	-----	+PTT-	T----	T----	T----	-----	T----	-----	-----	-----							
Barrow L 1		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Sold						
918	-----	PTT--	T----	T----	TT---	PT---	-----	-----	-----	-----							
Barrow L 3		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Sold						
922	-----	PT---	-----	T----	PT---	T----	PT---	++PT-	+++-	+++-	+++-	+PP--	PT---	P----	+PT--		
Boar L 4		Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
929	-----	-----	T----	T----	T----	-----	-----	-----	T----	-----	-----	-----	-----	Castrated 2-24-43 and sold		Testes Negative	
Boar L 5		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.				
941	-----	T----	PT---	-----	T----	-----	-----	-----	----	-----	-----	-----	PT---	Castrated 2-24-43 and sold		Testes Negative	
Boar L 7		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.				

[1] + = Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

[2] Normal, indicates the litter was normal and the cultures from fetal membranes, milk, and destroyed or dead pigs were negative for Br. suis to both direct culture and guinea-pig inoculation methods.

[3] Intradermal injections of Brucella extracts applied prior to this test.

TABLE 4—Results of Exposing Weanling Pigs to Brucella Suis per Vagina and Prepuce
(Date of exposure June 15, 1942)

Pig Sex Litter	Pre-Exposure Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after exposure) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Bacteriologic Results at Autopsy	Farrowing Results[2]
911	-----	-----	-----	T---	-----	TT--	TT--	++P-	++++-	++++-	++PP-[3]	+PPT-	+++P-	++P--	+PT--		
Female																	Normal
L 2		Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
914	-----	TT--	T---	TT--	T---	T---	T---	-----	-----	T---	++PT-[3]	P---	T---	T---	-----		
Female																	Normal
L 2		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
915	-----	TT--	T---	T---	PT--	+T--	PTT--	+PT--	PT--	++PT-	++PT-[3]	+PT--	+P--	PT--	+PT--		
Female																	Normal
L 2		Neg.	Pos.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
917	-----	-----	T---	T---	TT--	T---	T---	-----	-----	T---	++PT-[3]	+PT--	PT--	-----	-----		
Female																	Normal
L 2		Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
970	-----	+PT--	+PT--	PT--	++P--	++T--	PTT--	+PTT-	++P--	++PT-	++T--	+PT--	+P--	+P--	++PT-		Not
Female																	Pregnant
L unknown		Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
927	-----	TT--	T---	T---	T---	P---	T---	P---	TT--	T---	T---	-----	P---	Castrated		Testes	
Boar														2-24-43		Negative	
L 4		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	and sold			
933	-----	-----	-----	-----													
Boar					Destroyed 8-14-42											Negative	
L 5		Neg.	Neg.	Neg.													
960	-----	-----	TT--	T---	-----	+---	T---	-----	-----	-----	-----	-----	PT--	Castrated		Testes	
Boar														2-24-43		Negative	
L 9		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	and sold			
916	-----	T---	+P---	T---	PT--	+PT-[3]	++T--										
Boar							Destroyed 9-24-42									Negative	
L 2		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.										
971	-----	TT---	++P--	PTT--	PTT--	PP---	+P---	+P---	T---	+PT--							
Barrow											Sold						
L unknown		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.							

[1] + = Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

[2] Normal, indicates the litter was normal and the cultures from fetal membranes, milk, and destroyed or dead pigs were negative for Br. suis to both direct culture and guinea-pig inoculation methods.

[3] Intradermal injections of Brucella extracts applied prior to this test.

viously described. These gilts were bred using 4 of the original boars that had been artificially exposed to *Br. suis*. At the time of parturition, the fetal membranes, colostrum milk, and at least 1 live pig and all dead pigs from each litter were examined for *Br. suis* using both direct culture and guinea pig inoculation procedures.

RESULTS

Group 1 was composed of 4 females, 4 boars, and 2 barrows, exposed per rectum. The agglutination titers obtained ranged between incomplete at 1:50 and 1:400. *Br. suis* was isolated from the blood of 4 boars, 2 females, and 1 barrow on or before ninety-nine days after exposure. The titers of these 7 animals at the time of recovery of *Br. suis* from the blood were: 3, incomplete at 1:25 dilution; 3, incomplete at 1:100 dilution; and 1, incomplete at 1:200 dilution. *Br. suis* was recovered from the blood on 2 separate examinations in only 4 of these pigs. Two boars, 1 female, and 1 barrow were destroyed at 60, 162, and 171 days after exposure. *Br. suis* was recovered from the spleen, liver, and lymph nodes of 1 boar; from the lymph nodes and lungs of the female. The agglutination titers of these 2 pigs at the time of autopsy were incomplete in 1:100 and incomplete in 1:50 dilutions, respectively. Negative bacteriologic results were obtained from examinations of the tissues of the barrow and 1 boar. The 2 pigs from which organisms were not recovered carried titers of incomplete at 1:100. The details of results obtained in this group are shown in table 1. It will be noted that gilts 920 and 972 carried their pigs full term in normal pregnancies. Gilt 923 did not conceive.

Group 2 was composed of 4 females, 4 boars, and 2 barrows exposed *per os* on the feed. The agglutination titers obtained ranged between an incomplete reaction at 1:25 and 1:400 dilutions. *Br. suis* was isolated from the blood of 3 boars, 2 females, and 1 barrow on or before sixty-four days after exposure. The titers of these six animals at the time of the recovery of the organisms were 2 negative, 1 incomplete at 1:25, 1 incomplete in 1:50, and 2 incomplete in 1:100 dilutions. *Br. suis* was recovered from only 2 of these animals on 2 separate examinations of the blood. One female and 2 boars were destroyed at 15, 101, and 171 days after exposure. *Br. suis* was obtained from the lymph nodes, spleen, and kidneys of the female. Bacteriologic results were negative in both boars. The agglutination titers at autopsy were incomplete in 1:200 dilution for the female; negative in 1:25 and incomplete in 1:50 dilutions, respectively, for the boars. The results as indicated in table 2 show that gilts 907, 913, and 944 farrowed normally.

Group 3 was composed of 4 females, 3 barrows, and 3 boars exposed to *Br. suis* per the conjunctiva. The agglutination titers obtained ranged between an incomplete reaction in 1:25 and incomplete reaction in 1:200 dilutions. *Br. suis* was recovered from the blood of 2 females and 1 boar, sixty-four days after exposure. The titers of these 3 animals at the time of recovery of *Br. suis* were negative in 1:25 and incomplete in 1:25 and 1:50 dilutions, respectively. *Br. suis* was recovered from 2 of the 3 animals on 2 separate examinations of their blood. One barrow was destroyed 101 days after exposure with negative bacteriologic and agglutination results. Gilts 909, 926, 935, and 948 completed normal gestation periods. Boars 929 and 941 were castrated and the testicles examined for *Br. suis* with negative results. Detailed results obtained with this group are given in table 3.

Group 4 was composed of 4 boars, 1 barrow, and 5 females exposed per prepuce and per vagina. The titers obtained ranged between negative in 1:25 to incomplete in 1:200 dilutions with the highest titers in the females. Bacteriologic examinations of the blood were negative in the boars and the barrows and 1 of the females. *Br. suis* was isolated from the blood of 4 of the females on or before eighty-four days after exposure. Results of bacteriologic examinations were positive on 2 separate examinations of 2 of the females. The titers of these 4 females at the time of recovery of *Br. suis* from the blood were: 1 negative, 1 incomplete in 1:25, 1 incomplete in 1:50,

TABLE 5—Results of Exposing Weanling Pigs to Brucella Suis Subcutaneously
(Date of exposure June 15, 1942)

Pig Sex Litter	Pre-Exposure Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after exposure) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Bacteriologic Results at Autopsy	Farrowing Results[2]
906	-----	++++P	+++PT	+++PT	+++P-	+++--											
Female							Destroyed 9-24-42									Negative	
L 1		Neg.	Pos.	Neg.	Neg.	Neg.											
912	-----	++PT-	+++PT	+++T--	+++PT-	+++T-	+++PTT	+++P--	+P---	+----	+++PT-	+P---	+----	P----	+PT--		
Female																	Normal
L 2		Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
937	-----	+++PT	+++PTT	+++PT	+++PT	++++T	+++PTT	+++PT-	+PT--	+++T--	+P---	T----	T----				
Female														Destroyed			Normal
L 6		Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	3-30-43			
943	-----	+++PT	+++P-	++++P	+++P-	++++T	+++PTT	+++TT	+++PT-	+PT--	+++P--	+PT--	+----	PT---	+PT--		
Female																	Normal
L 7		Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
966	-----	+++PT	+++PT	++++P	++++P	+++++	+++PP	++++T	+++PT	++++P	+++P-[3]	+++P-	+++P--	+P---	+PT--		
Female																	Normal
L unknown		Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.	Neg.		
924	-----	+++PT															
Boar			Died 7-1-42													Positive	
L 4		Neg.															
931	-----	+++P-	+++PT	+++T-	+++PT-	++++P	+++PTT	+++TT	+++P--	+++T-							
Boar											Destroyed 12-3-42					Negative	
L 5		Neg.	Pos.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.							
950	-----	+++PT-	+++T-	+++PT-	+++P-	+++PT	+++PT	+++P-	PTT--	+++PT-	PT---	T----	PT---	Castrated		Testes	
Boar														2-24-42		Negative	
L 8		Pos.	Pos.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	and sold			
958	-----	+++--	+PT--	T----	+++PT-	+++T-	+++PT	+++PT-	+T---	+++P--	-----	P----	P----	Castrated		Testes	
Boar														2-24-43		Negative	
L 9		Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	and sold			
965	-----	+++P--	+++PT-	+++PT	++++P	++++P	+++PT	++++T	+++PP-	+++P--							
Barrow											Destroyed 11-24-42					Positive	
L unknown		Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.							

[1] + = Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

[2] Normal, indicates the litter was normal and the cultures from fetal membranes, milk, and destroyed or dead pigs were negative for Br. suis to both direct culture and guinea-pig inoculation methods.

[3] Intradermal injections of Brucella extracts applied prior to this test.

TABLE 6—Results of Exposing Weanling Pigs to Brucella Suis Intravenously
(Date of exposure June 15, 1942)

Pig Sex Litter	Pre-Exposure Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after exposure) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Bacteriologic Results at Autopsy	Farrowing Results[2]
903 Female L 1	-----	+++PT Pos.	+++PT Pos.	++++P Neg.	++PT- Pos.	++++T Neg.	++++P Pos.	++++P Neg.	++++P Neg.	++P- Neg.	++PT-	+PT--	++PT- Neg.	+P--- Neg.	+PT-- Neg.		Normal
940 Female L 7	-----	+++PT Neg.	+++PT Pos.	+++PP Neg.	+++P- Pos.	+++++ Neg.	+++PT Neg.	++++P Neg.	+++PT Neg.	+++T- Neg.	++PT-	++P--	++--- Neg.	+P--- Neg.	+P--- Neg.		Normal
947 Female L 8	-----	+++PT Neg.	+++PT Pos.	++++P Neg.	++++P Neg.	+++PT Neg.	+++P- Neg.	+++T- Neg.	+PT-- Neg.	+++P- Neg.	+PT--	P----	----- Neg.	PT--- Neg.	PT--- Neg.		Normal
955 Female L 9	-----	+++PT Neg.	+++PT Pos.	+++T- Neg.	+++P- Neg.	++++P Neg.	++++P Neg.	+++P- Neg.	++P-- Neg.	++T-- Neg.	+++T-[3]	+---	T---- Neg.	----- Neg.	T---- Neg.		Normal
973 Female L unknown	-----	+++PT Neg.	+++PT Pos.	+++PT Neg.	Destroyed 8-14-42											Positive	
910 Barrow L 2	-----	+++PT Pos.	++P-- Neg.	+++T- Neg.	++PT- Neg.	+++T- Neg.	++PT- Neg.	+++T- Neg.	++T-- Neg.	++PT- Neg.	Destroyed 11-24-42					Negative	
919 Barrow L 3	-----	++++P Pos.	+++PT Pos.	++++- Neg.	+++++ Pos.	+++++ Neg.	++++P Pos.	+++++ Neg.	+++++ Neg.	+++++ Neg.	Destroyed 11-24-42					Positive	
962 Barrow L unknown	-----	+++PT Neg.	+++PT Neg.	++++P Neg.	++++P Pos.	++++P Neg.	+++PT Neg.	+++T Neg.	++PT- Neg.	+++P- Neg.	Destroyed 11-24-42					Negative	
921 Boar L 4	-----	++++P Pos.	+++PT Pos.	+++PT Neg.	+++PT Pos.	+++PT Neg.	+++P- Neg.	+++P- Neg.	++PT- Neg.	+P--- Neg.	+T--- Neg.	T---- Neg.	P---- Neg.	Castrated 2-24-43 and sold		Testes Negative	
928 Boar L 5	-----	+++PT Pos.	+++PTT Pos.	+++PT Neg.	+++P- Pos.	+++PT Neg.	+++PTT Neg.	+++P- Neg.	+++PTT Pos.	+++++ Neg.	Destroyed 12-3-42					Positive	

[1] + = Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

[2] Normal, indicates the litter was normal and the cultures from fetal membranes, milk, and destroyed or dead pigs were negative for Br. suis to both direct culture and guinea-pig inoculation methods.

[3] Intradermal injections of Brucella extracts applied prior to this test.

and 1 incomplete in 1:100 dilutions. Two boars carrying titers of negative in 1:25 and incomplete in 1:50 dilutions, respectively, were destroyed at 60 and 102 days after exposure. Bacteriologic examinations of the tissues were made with negative results. Boars 927 and 960 were castrated and the testicles examined for *Br. suis* with negative results. Gilts 911, 914, 915, and 917 completed normal pregnancies. Gilt 970 did not conceive. Detailed results obtained with this group are given in table 4.

Group 5 was composed of 5 females, 4 boars, and 1 barrow exposed to *Br. suis* subcutaneously. All developed titers of incomplete in 1:400 dilution on or before ninety-nine days after exposure. *Br. suis* was recovered from the blood of all but 1 boar, which died from extraneous causes fifteen days after exposure. These results were obtained on or before sixty-four days after exposure in all cases. *Br. suis* was recovered from the blood of 6 pigs only once, with 2 or more such isolations from 3 of these pigs. All pigs from which organisms were recovered carried titers of 1:100 or higher at the time of isolation of the bacteria. Five pigs in this group were destroyed at 15, 101, 162, and 171 days after exposure. *Br. suis* was obtained from the tissues of 2 which were carrying titers incomplete at 1:100 and 1:400 dilution at the time. Negative results were obtained from bacteriologic examinations of the other 3 pigs which were carrying titers in 1:25, 1:100, and 1:200 dilutions at the time. Boars 950 and 958 were castrated and the testicles examined for *Br. suis* with negative results. Gilts 966, 912, 937, and 943 completed normal pregnancies. Gilt 937 was destroyed after farrowing. Results of bacteriologic examinations of the tissues were negative. Detailed results obtained with this group are given in table 5.

Group 6 was composed of 5 females, 2 boars, and 3 barrows exposed to *Br. suis* intravenously. All developed titers of incomplete in 1:400 (maximum dilution employed) on or before eighty-four days after exposure. *Br. suis* was isolated from the blood of all pigs in this group on or before sixty-four days after exposure. The organism was recovered from the blood for variable lengths of time as follows: from fifteen to sixty-four days in 1, thirty to sixty-four days in 1, fifteen to ninety-nine days in 2, and from 15 to 128 days in 1. Organisms were recovered at one examination only in the remaining 5 pigs. The titers at the time of the first recovery of the organism from the blood were incomplete in 1:400 (maximum dilution employed) in all pigs. Five pigs were destroyed, 1 at sixty days, 3 at 162 days, and 1 at 171 days after exposure. *Br. suis* was recovered from the tissues of 1 female, 1 barrow, and 1 boar, and negative results were obtained in 2 barrows. The titers of these pigs at autopsy were incomplete in 1:200 dilution or higher. Boar 921 was castrated and the testicles examined for *Br. suis* with negative results. Gilts 903, 940, 947, and 955 completed normal pregnancies. It is interesting to note that boar 928 yielded *Br. suis* from the seminal vesicles at autopsy one month after eliminating a semen sample from which *Br. suis* was not recovered with the technique employed. Detailed results obtained with this group are given in table 6.

The results of holding Brucella-free pigs in close proximity to pigs artificially exposed as described above are set forth in table 7. These pigs apparently did not become infected as evidenced by negative bacteriologic and essentially negative agglutination results. The boars were castrated at 7 months of age and sold as stags. Bacteriologic examinations of the testicles were all negative. The 3 females completed normal pregnancies.

DISCUSSION

Br. suis was recovered from 8 of 18 artificially exposed pigs brought to autopsy for examination of the tissues. Also, such organisms were recovered from the blood of 40 of the 60 exposed pigs and agglutinins were demonstrated in 1:25 dilution or higher at some time in the course of the experiment in the blood of all but 1 of these pigs. Thus, these experiments seem to demonstrate that

TABLE 7—Results of Holding Weanling Pigs in Close Proximity to Exposed Pigs
(Placed on experiment June 15, 1942)

Pig Sex Litter	Pre-Experiment Titer	Agglutination Titers[1] and Blood Cultural Results (Time in days after placed on experiment) 15	30	49	64	84	99	112	128	144	185	220	269	311	339	Farrowing Results[2]
952	-----	-----	-----	-----	TT---	T----	T----	T----								
Boar									Sold							
L 8						Neg.	Neg.	Neg.								
956	-----	T----	-----	-----	T----	-----	-----	PT---								
Boar									Sold							
L 8						Neg.	Neg.	Neg.								
946	-----	-----	T----	-----	T----	-----	TT---	PT---	-----	T----	T----	-----	-----	PT---		
Female																Normal
L 7						Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.		
961	-----	T----	-----	-----	T----	-----	-----	-----								
Boar									Sold							
L 9						Neg.	Neg.	Neg.								
945	-----	-----	-----	-----	-----	T----	-----	T----	-----	-----	-----	T----	P----	T----		
Female																Normal
L unknown						Neg.	Neg.	Neg.	Neg.	Neg.			Neg.	Neg.		
964	-----	TT---	T----	T----	TT---	TT---	-----	-----	-----	-----	T----	-----	-----	T----	PT---	
Female																Normal
L unknown						Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		Neg.	Neg.	Neg.	
953	-----	-----	-----	T----	T----	T----	TT---	TT---								
Boar									Sold							
L 8						Neg.	Neg.	Neg.								
939	-----	-----	-----	-----	T----	-----	-----	-----								
Boar									Sold							
L 6						Neg.	Neg.	Neg.								
942	-----	-----	-----	-----	T----	-----	-----	-----								
Boar									Sold							
L 7						Neg.	Neg.	Neg.								
957	-----	-----	-----	-----	T----	-----	-----	-----								
Boar									Sold							
L 9						Neg.	Neg.	Neg.								

[1] + = Complete agglutination; P = Partial agglutination; T = Trace of agglutination; - = No agglutination.

[2] Normal, indicates the litter was normal and the cultures from fetal membranes, milk and destroyed or dead pigs were negative for Br. suis to both direct culture and guinea-pig inoculation methods.

a high percentage of heterogeneous pigs of weanling age can be readily infected with *Br. suis* organisms through various channels as measured by the agglutination and bacteriologic results. Conjunctival and prepuce exposures appear to be the least satisfactory of any of the channels of exposure used on weanling pigs in these experiments. It is, of course, quite possible that the groups exposed per rectum, conjunctiva, and vagina and prepuce were also exposed to secondary oral infection from a contaminated environment.

All of 21 gilts exposed to *Br. suis* as weanling pigs carried their pigs to full term in their first pregnancies even though they were carrying significant agglutination titers at the time they were bred. If these results are repeatable, they may provide at least a partial explanation as to why some naturally infected herds of swine have sieges of abortion while other infected herds do not show this symptom. In other words, these results may point to a part of the natural course of the disease by indicating what follows infection of gilts with *Br. suis* acquired at or near weaning age.

In no case were *Br. suis* organisms recovered from the fetal membranes or colostrum milk of these gilts or any of the tissues of their newborn pigs at the time of parturition. This is strikingly different from results usually obtained from Brucella-infected cows. If these results can be repeated, they have important implications concerning control procedures because apparently these artificially exposed weanling gilts were not a source of *Br. suis* infection at the termination of their first pregnancies.

Many of these gilts were carrying a relatively high agglutination titer in the colostrum milk at the time of farrowing, but in no case were specific agglutinins demonstrated in the blood of newborn pigs before nursing. However, in as short a time as six hours after nursing, some of the pigs showed a low titered agglutination response. This low titered agglutination response in such pigs disappeared rather rapidly after weaning and in many cases disappeared before weaning. This seems to indicate that there is frequently a concentration of agglutinins in the colostrum of such gilts which subsequently disappears from the milk of the sow during the suckling period; hence, some such suckling pigs no longer carry an agglutination reaction. This indicates that extreme care may be necessary in interpreting the agglutination test in young weanling pigs, since it is likely that positive agglutination reactions in these pigs are the result of absorption of agglutinins from the milk of the mothers and not evidence of active infection. It should be pointed out again that *Br. suis* was not recovered from any of these gilts at the time of parturition. Mingle[19] has evidence which indicates that different agglutination results were obtained in pigs born from mothers that were eliminating *Br. suis* at the time of parturition. Thus, it may be that knowledge of the infection status of the mother at the time of parturition may be essential for a correct interpretation of agglutination reactions in weanling pigs.

These results suggest that agglutination methods as now employed for brucellosis in swine are adequate as a herd diagnostic procedure but may not be sufficiently reliable as a basis of diagnosis of brucellosis in individual swine. In several cases, it was observed that *Br. suis* was obtained from the blood as long as two months prior to the time that significant specific agglutinins could be demonstrated in the blood. It seems likely that animals that are carrying the organisms in the blood stream with possible localization in their body tissues without a significant agglutination titer could be a menace to the remainder of the herd, but would not be taken into consideration in any control program based on the agglutination test. In our field experiments, the practice of attempting to assemble a herd of swine free from brucellosis by purchases based on negative agglutination tests of individual animals from infected herds has failed.

SUMMARY

1) These experiments demonstrated that a high percentage of heterogeneous pigs were readily infected at weaning age with

Brucella suis as determined by agglutination and bacteriologic results. On this point, these results disagree with those of several previous investigations which have led to the common conception that such pigs are not readily infected artificially with *Br. suis* organisms.

2) These results indicate that the agglutination method, as now employed for brucellosis in swine, is adequate as a herd diagnostic procedure but may not be sufficiently reliable as a basis of diagnosis in individual swine.

3) Gilts infected with *Br. suis* as weanling pigs at about 12 weeks of age failed to show the symptom of abortion at the termination of their first pregnancies and *Br. suis* was not recovered from them or the tissues of some of their newborn pigs at the time of parturition.

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A Comparative Study of Two Biologic and Two Chemical Techniques of Pregnancy Diagnosis in the Mare

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BESIDES indicating the presence or absence of pregnancy so that necessary rebreeding may be possible, the methods of pregnancy diagnosis may be utilized to detect sterile males or females. Methods are available also for the detection of pregnancy in certain animals (sow and mare) both during the early and late stages of gestation, and these methods are of value in studying the resorption of embryos or the later fetal abortions. Interest in the latter instigated an investigation of two biologic and two chemical techniques for the detection of pregnancy in the mare. The mare is especially suitable for endocrinological investigations, because during pregnancy she produces and excretes in the urine in high concentration, six or more free and conjugated estrogens; and, in addition, produces a gonadotrophin which is retained in high concentration in the blood.

During this investigation, from one to three of four diagnostic techniques were applied to blood and urine specimens collected from 576 individual mares at different stages of the gestation period. However, it is the purpose of this paper to report the results of a two-year study of the comparative reliability of two chemical and two biologic techniques utilizing blood and urine specimens from approximately 100 mares on which frequent examinations were made and complete records were available for the entire gestation period. These mares were located at the United States Range Experiment Station, Miles City, Montana;* the U. S. Morgan Horse Farm, Middlebury, Vermont;† and the Animal Disease Research Center, Beltsville, Md.†

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*The author is especially indebted to Dr. F. N. Andrew, now at Purdue University, for making the specimens from Miles City available, for supplying the foaling data, and for his interest and aid during the investigation.

†Appreciation is extended to the U. S. Morgan Horse Farm and the Animal Disease Research Center for their coöperation in making urine specimens available and in supplying necessary foaling records.

METHODS

The following four techniques were used in this investigation: a biologic blood-serum test for the presence of gonadotrophin, a biologic test for estrogens in the urine, and two chemical techniques for the detection of urinary estrogens.

Blood-Serum Method.—Hart and Cole,[1] Saunders and Cole,[2] Day and Rowlands,[3] and others have adequately described and discussed the preliminary procedures involved in the collection and treatment of mare serum, its injection into immature rats, and the age of the rats used as assay animals. The method used in this laboratory for the past seven years is based upon the recommendations of Cole and his colleagues but includes modifications indicated by experimental results.

The blood is collected with a large bore needle in the usual manner, let stand at room temperature for an hour to facilitate the separation of serum, and then placed in a refrigerator. After the serum has separated, it is poured off and stored in the refrigerator. Three immature female rats (21 to 24 days of age) may be used but it is safer to use at least 6 divided into 3 groups. One group is kept as a control, the second group is injected with 0.5 cc. of serum, and the third group with 1.5 cc. of serum. If the serum has been collected from mares fifty to ninety days following the date of breeding, one can be quite certain that a positive reaction in the test animal receiving the smaller quantity of serum definitely indicates the state of pregnancy; whereas a negative response with the larger quantity of serum is diagnostic of the non-pregnant state.

The rats are examined after five days for increase in size of the ovaries and uterus over that of the controls and for the presence of follicles and corpora lutea.

Urine Method (Biologic).—At least 50 cc. of urine is collected from the mare after the third or fourth month of the gestation period. To 50 cc. of the urine, sulphosalicylic acid* is

*The sulphosalicylic acid is prepared by mixing H_2SO_4 and salicylic acid to form a thick paste.

added until the reaction with urinary carbonates ceases, then a slight excess (1 Gm. or less) is added. The acidified urine is agitated, allowed to stand thirty minutes, neutralized with Na_2CO_3, and filtered to remove precipitated substances which might prove toxic to the test animals.

The test animals are mature female rats castrated at least two weeks prior to injection. The castrated animals require occasional injections of estrogens to determine their sensitivity and those not used frequently must be injected with 1 r. u. of estrogen one week before injection of the urine specimen in order to obtain optimum results.

The usual procedure is to use 5 to 6 rats in two cages for each specimen. Each cage is used as a unit, each rat within a cage receiving the same quantity of urine—0.1 cc. per rat for one cage and 0.6 cc. per rat for the other—injected three times daily for two days. A vaginal smear is made at the time of the first injection and then twice daily for two days beginning the day following the two-day injection period. A positive reaction is a vaginal smear with an abundance of cornified cells and very few or no leucocytes and is indicative of pregnancy if obtained with smears from 2 or more rats receiving the smaller quantity of urine. The injection of the larger quantity of urine makes a negative reaction a more definite indication of the nonpregnant state.

The Cuboni Chemical Method.—This rapid and simple technique developed by Cuboni[4] and applicable to pregnancy diagnosis in the mare or the sow has been given previously in diagrammatic form and described in detail by Roth, Mayer, and Bogart.[5] Especial attention must be given to the recommendations for the removal of urinary pigments in order to secure the best results with this method.

The Phenolsulphonic Acid Method.—The author developed a chemical method for the diagnosis of pregnancy in the mare which is based on the Kober colorimetric test for estrogens[6] and on subsequent investigations of the test by Cohen and Marrian.[7] Although the Kober test in which estrogens produce a characteristic cherry red color when heated with a phenolsulphonic acid reagent has been used by numerous investigators for the quantitative estimation of estrogens, only two previous investigators have used this test for the diagnosis of pregnancy, Patterson[8] (in man) and Kober†[9] (in the mare). The adaptation of the Kober test developed in this laboratory utilizes the wide divergence in estrogen concentration in the urine of nonpregnant and pregnant mares for diagnostic differentiation.

On the basis of the results of numerous experiments made to ascertain the exact volume of urine and the optimum conditions of hydrolysis, extraction and final color formation required for accurate pregnancy diagnosis, the following procedure was adopted:

The hydrolysis of the conjugated estrogens is accomplished by slowly adding 3 cc. of concentrated HCl to 15 cc. of a specimen‡ of mare's urine in a Pyrex test tube and heating in a boiling water bath for ten minutes. This is a modification of the hydrolysis technique recommended by Smith and Smith.[10] Following the hydrolysis, the urine is cooled to 15 C. in an ice water bath to precipitate urinary pigments and substances formed during hydrolysis which mask the final red color. The precipitated substances are removed by filtration and the amber-colored filtrate collected in a separatory funnel.

The filtrate in the funnel is extracted twice with 20 cc. and once with 10 cc. of peroxide-free ether to remove the estrogens. The combined ether extracts are then shaken in the separatory funnel with 10-cc. portions of 20 per cent Na_2CO_3 solution until the carbonate washes are colorless (usually three or more washes are required). The carbonate washes are followed by a final wash with 20 cc. of distilled water.

The ether extract at this stage is nearly colorless but, in many instances, may contain a purplish-red pigment. The washed ether is now extracted with three 15-cc. portions of N/10 NaOH in which the estrogens are soluble but in which many of the ether-soluble, contaminating substances do not dissolve. The NaOH extracted ether is washed once with 15 cc. of distilled water. The water wash and three NaOH extracts are combined, and 25 per cent H_2SO_4 added until the solution is acid to congo red paper. The combined acidified N/10 NaOH extracts and water wash are then extracted with ether to remove estrogens as previously described for the filtrate of the urinary hydrolysate. The 20 per cent Na_2CO_3 and water washes are also made if the ether extracts are brown.

The three ether extracts of the acidified NaOH solution are poured into a clean dry 125 cc. Erlenmeyer flask preventing, however, the inclusion of water adhering to the separatory funnel. After the evaporation of the ether in a water bath, 3 cc. of a specially prepared phenolsulphonic acid reagent§ are added and the contents of the flask heated for five minutes in a boiling water bath. The

†A quantitative method in which B-Naphthol was substituted for phenol in the color-producing reagent.

‡The specimen may be taken from urine voided at any time or urine collected by catheter, but contamination must be prevented.

§To 3.6 parts of freshly distilled phenol, 5.6 parts of H_2SO_4 are slowly added. The flask containing the phenol is rotated in an ice water bath during and for fifteen minutes after the addition of the acid.

flask is immediately plunged into an ice water bath and cooled. To the cooled mixture, 3 cc. of a 5 per cent H_2SO_4 solution are added, the flask again heated for a three minute period, cooled, and diluted to 10 cc. by the addition of 4 cc. of water or 5 per cent H_2SO_4.

The formation of a pink to cherry red color, the intensity of the color depending upon the stage of pregnancy when the urine specimen was collected, constitutes a positive test for pregnancy. If the procedure has been carefully followed, no interfering colors should appear. The addition of a few drops of Superoxal (30 per cent hydrogen peroxide) and warming (Kober[6]) causes all of the color produced by the conjugation reaction between estrogens and the reagent to disappear but does not affect "nonestrogen" colors. The H_2O_2 reaction, thus, can be used as a confirmatory test.

The nonpregnant state is indicated when the residue of a urine extract does not form the red color with the reagent.

RESULTS

Biologic Versus Chemical Methods.—During the fall of 1937, a total of 97 urine specimens were received from the three government research centers. The specimens were immediately designated as indicating the presence or absence of pregnancy in the mares on the basis of the results of the three techniques: biologic, Cuboni, and phenolsulphonic acid, each dependent upon the presence of estrogens in the urine. The results were recorded and compared at a later date (usually nine to twelve months later) with the foaling records of the individual mares.

On 66 mares reported to have foaled, all three urine methods—a biologic and two chemical methods—gave a positive diagnosis in every case with specimens collected from the 120th to the 250th day of the gestation period. Hence after the 120th day, all three methods were equally reliable. However, in the cases of 2 mares, urine specimens collected earlier in pregnancy were available. In 1 of these, the urine was collected on the eighty-fifth day and both chemical methods gave a negative result, whereas the biologic test was positive, indicating that the biologic method may give a positive reaction at an earlier stage of pregnancy than the chemical methods. In the second, in which the specimen of urine was collected on the ninety-first day, the Cuboni method gave a negative reaction while the biologic and the new chemical method gave positive reactions, suggesting that both may be more sensitive than the Cuboni and that the new chemical technique will give a positive reaction as early as the ninety-first day. In both cases, urine specimens collected after the 120th day gave positive diagnoses with all three urine methods. Subsequent investigations on specimens not herein reported confirm the general order of sensitivity of the three methods; but, because of individual variation in the mares, the earliest positive reaction did not always occur at exactly the time reported for the 2 cases described.

On urine specimens from the mares reported as nonpregnant, the results were less reliable. The reason for this unreliability will be discussed later. A total of 28 mares did not foal, but only on 24 of these did all three methods give a negative diagnosis of pregnancy (85.7 per cent correct by agreement of the three methods

TABLE 1—Comparative Reliability of the Biologic, Cuboni and Phenolsulphonic Acid Methods of Pregnancy Diagnosis on the Urine of 97 Mares*

		Biologic Method			Cuboni Method			Phenolsulphonic Acid Method		
Samples	Foaling record	No. +	No. —	% correct	No. +	No. —	% correct	No. +	No. —	% correct
66	all foaled	66	0	100	66	0	100	66	0	100
28	none foaled	4	24	85.7	1	27	96.4	1	27	96.4
3	no record**	1	2	...	1	2	...	1	2	...

*Urine specimens obtained after 120th day of gestation period.
**Mares died before parturition.

with the foaling data). In 1 of the other 4 mares, abortion may have occurred since she produced no foal in spite of a positive pregnancy diagnosis by all three methods. The results of the urine specimens from the other 3 mares suggest that the biologic is a less reliable test than the two chemical methods. In 1 case, the biologic test was doubtful and in 2 cases positive, while both chemical methods were negative in all 3 mares; the biologic test giving correct diagnoses in but 85.6 per cent of the nonpregnant mares; the chemical diagnoses being correct in 96.4 per cent of these mares. The results of this phase of the investigation are summarized in table 1.

Comparison of Blood-Serum Test with the Cuboni Test and Foaling Records.—The blood-serum method of pregnancy diagnosis is applicable to serums of animals of the genus Equus since a gonadotrophin is present in appreciable quantities in their blood during pregnancy. This method is possibly used most frequently because a positive diagnosis can be obtained early in the gestation period. A total of 71 mares was diagnosed by the serum method and foaling records were available on 69 of them. A Cuboni test was run on 29 of the 69 mares.

The blood-serum method diagnosed 37 of the 69 mares as pregnant, but 45 actually foaled. It, then, was correct in 82.2 per cent of the mares which subsequently foaled. This method diagnosed as nonpregnant 18 of the 22 mares reported to be without foal—a percentage of correct diagnoses by the blood-serum method of 81.8. The percentage of correct diagnoses, both in the pregnant and the nonpregnant mares in this particular group, is exceedingly low and not in agreement with results we usually obtain with the serum test nor with results reported in the literature. Reasons possibly accounting for the low percentage of correct diagnoses are presented and discussed later.

Again the Cuboni test was 100 per cent correct in diagnosing pregnancy in 21 mares which later were reported as having foaled. This chemical method, however, diagnosed 3 of 8 reportedly nonpregnant mares as pregnant. Interestingly, though, the blood-serum test, run several months prior to the Cuboni, also diagnosed these same mares as pregnant. Possibly these were unreported abortions as the mares in question were range mares, examined not more than once daily and in many cases less often.

These positive reactions with both blood and urine specimens from mares which did not subsequently foal are not the only paradoxical results accumulated on this series of 69 mares. There were 8 mares on which the serum tests were negative, the Cuboni tests were positive, and all 8 mares foaled. Another mare reported in the nonfoaling group was diagnosed as pregnant by the serum test and negative by the Cuboni test several months later.

DISCUSSION

The economy of the chemical methods of pregnancy diagnosis has long been recognized, but, apparently, their comparative reliability has not been so well recognized. The superior results obtained with these methods on the specimens reported in this investigation, on specimens from several hundred other mares not included in this paper, and those obtained by other investigators using the Cuboni method (Romano,[11] Svensson,[12] Cuboni[13,14,15]) are evidence of the reliability of the chemical methods and have led this laboratory to use them in place of the biologic methods on all urine specimens received during the past two years. A positive reaction with either of the chemical techniques on a specimen of mare urine can be accepted as a definite indication of the state of pregnancy. The results of previous investigators using the Cuboni and the two biologic methods are in agreement with results reported in this paper in showing a high percentage of correct diagnoses on the mares which subsequently foal.

The negative reaction is equally reliable in indicating the absence of pregnancy if certain provisions have been recognized and employed. Some negative reactions are obtained on specimens from mares which eventually foal because the breeding date has been disregarded and the specimen col-

lected too early in the gestation period to obtain a correct diagnosis with the method employed. Since individual animals differ in the concentrations of hormones in the blood or urine at identical stages in pregnancy, the optimum time for the collection of specimens is that at which the peak concentration of hormones has been reached by those individuals which lag in this respect. A large number of negative, blood-serum tests is indicative of poor fertility in the stallion used during the breeding period, of early abortions, or (and this should be carefully considered before conclusions are drawn) of insufficient care given to the collection and storage of the serum and to the technique followed in making the test.

A low percentage of correct diagnoses has been reported repeatedly in the literature—and the results of this investigation corroborate these reports—on mares which fail to foal. An important consideration that may possibly explain these incorrect diagnoses is the question of abortion. In the series of mares studied in this investigation, 8 were reported as having failed to deliver a foal, yet 3 were diagnosed as pregnant by the blood-serum method and several months later were similarly diagnosed by the Cuboni technique on a urine specimen. It may be that these mares had conceived and then had aborted between the date of collection of the latter specimen and the time parturition should have occurred. The serum of 1 of the 8 reportedly nonfoaling mares gave a positive reaction indicating pregnancy, but later the urine reacted negatively. In this case, abortion might possibly have occurred between the dates on which the 2 specimens were obtained. In a large group of mares, then, the use of the early serum test, one of the later urine tests, and a careful consideration of foaling data may not only indicate abortions but, in addition, may indicate the approximate time of their occurrence.

As previously stated, the percentage of correct diagnoses by the blood-serum method was much less than the 95 per cent or above usually obtained. Although the serum specimens were shipped to the author during the late summer and early autumn months, with no precautions to protect them from the high temperature, it is our opinion that the poor results were not caused by high environmental temperatures during shipment, as several hundred other serum specimens shipped under similar conditions have yielded a percentage of correct diagnoses above 95 per cent. Since the majority of the mares used in this investigation were range mares, and could not be carefully watched for abortions, 2 of which were reported and 8 or more indicated by the results, it might be that unreported abortions occurring early in the gestation period influenced the correctness of the results with the serum method.

SUMMARY AND CONCLUSIONS

A comparative investigation of the reliability of a biologic technique and two chemical techniques—the Cuboni and a new test adapted by the author—indicates that after the third month of the gestation period all three urine methods are equally reliable in the diagnosis of pregnancy in mares which later foaled.

However, in mares reported as not foaling, the two chemical methods gave correct diagnoses on 96.4 per cent of the specimens examined whereas the biologic test was less accurate, giving but 85.7 per cent negative reactions on these mares. The relative unreliability of the methods of pregnancy diagnoses with mares which fail to foal is discussed and possible explanations for this unreliability and its bearing on the problem of resorption and abortion are presented.

The blood-serum test was the least accurate of any of the four employed, yielding but 82 per cent correct diagnoses in both pregnant and nonpregnant mares. The unusual inaccuracy of the serum method in this investigation has been discussed and possible explanations for it presented.

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Bird Pox in the Sooty Grouse, Dendragapus Fuliginosus Fuliginosus, with Recovery of the Virus

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THE IMPORTANCE of avian pox as a disease of birds has been recognized ever since the pathogenicity of the causal agent of fowl pox (contagious epithelioma of fowl) was first demonstrated by Bollinger.[1] Since then, bird pox is reported to have occurred under natural or experimental conditions in a wide variety of birds (for reviews, *see* [2,3]).

Avian pox is commonly considered to fall into three groups, namely: fowl pox, pigeon pox, and canary pox. These groups, however, are not clearly defined,[3-7] whether they be considered from the point of view of host susceptibility or immunologic relationships. Conclusive evidence for the occurrence of fowl pox as shown by both characteristic pathologic changes and recovery of the virus is limited to domesticated birds, rarely affecting wild birds reared in close proximity to barnyard fowl. Accordingly, the discovery in wild fowl of bird pox as a natural infectious disease is noteworthy. It is the purpose of the present paper, therefore, to report the recovery of the virus of bird pox from the sooty grouse, *Dendragapus fuliginosus fuliginosus*, and to record experiments that establish its identity as the virus of bird pox.

HISTORY OF THE NATURAL OUTBREAK

In the autumn of 1939, many sooty grouse (*Dendragapus fuliginosus fuliginosus*) inhabiting the Cowichan Valley of southeastern Vancouver Island were reported by hunters to be diseased. Of 5 grouse shot out of a single covey from this area, 3 were sent to one of us (I. M. C.) for examination and the other 2 were left where they fell because their heads and necks were so extensively covered by lesions that the hunter was hesitant to handle them.[8] The grouse were found to have wartlike tumors involving the periorbital tissues, sometimes completely covering the eyes (fig. 1). Histologic study of these tumors led to a diagnosis of low grade papillary carcinoma.[8] The sections showed well-differentiated, squamous epithelial cells which had not infiltrated the deeper tissues (fig. 2). These findings made it clearly desirable to obtain additional material for virus studies.* Accordingly, during 1940, 1,300 sooty grouse were examined, but the disease was not detected. However, of the 1,800 sooty grouse examined during the 1941 hunting season, 4 affected birds were found. Two of these birds were obtained in the same region as that from which the original birds had come (about 12 miles west of Duncan, B. C.), and the other 2 were obtained from a region within 30 miles. The affected birds were from the 1941 hatch. The lesions on these birds were more widely distributed than on those discovered in 1939, but were confined to glabrous and sparsely feathered areas. The largest growths involved the periorbital skin, but they also extended to the base of the beak, obstructed the nostrils, and in one instance invaded the epithelium of the hard palate, preventing normal closure of the beak. Smaller growths were present on the crown and nape and on the lower leg including the heel, tarsus, and interdigital skin. Gross dissection and microscopic examination revealed no involvement of the underlying osseous tissue. Hyperplastic epithelial tissue was removed

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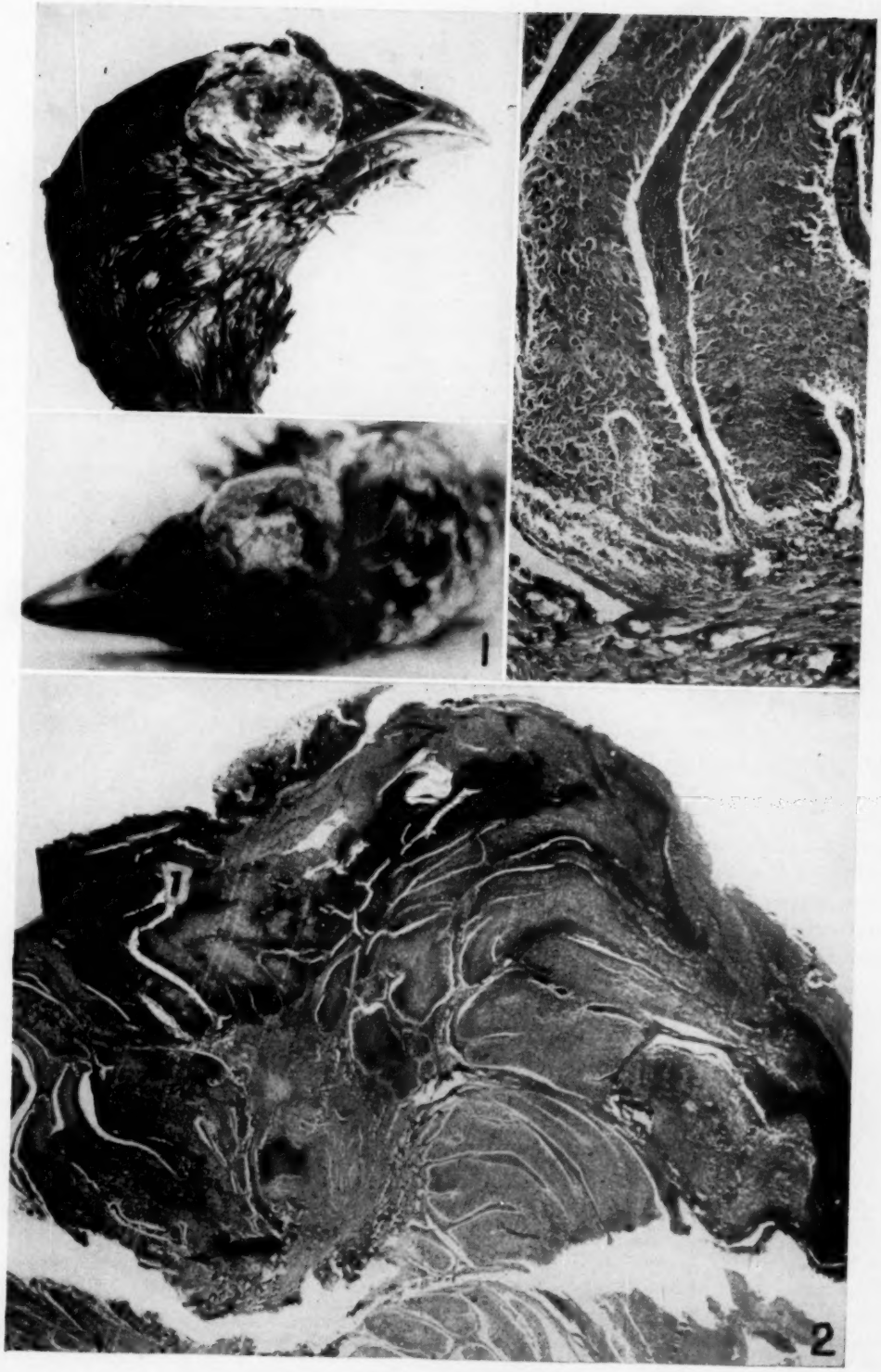


from these 4 birds for histologic and virus studies.

MATERIALS AND METHODS

The tissues under investigation consisted of epithelial tumors removed from 4 grouse. They were sent to Rochester in 50 per cent glycerol. For purposes of comparison and confirmation, fowl-pox virus of known properties was employed. Dr. Ernest W. Goodpasture† kindly sent us the fowl-pox virus contained in glycerinated chorioallantoic tissues of developing chicks.

A single method of preparation was employed, both to make suspensions of tissue representing the disease under investigation, and the tissue containing fowl-pox virus for testing immunity. When tissues had been stored in 50 per cent glycerol, these tissues were washed three times in Locke's solution. Then the washed tissue, or more usually fresh tissue, was triturated with an abrasive,‡ diluted with Locke's solution to yield a 10 per cent suspension, and centrifuged at 1,500 r.p.m. for thirty minutes. The supernatant fluid, without further dilution, was used as source virus for inoculating white Leghorn chicks that ranged in age from 12 hours to 6 days. The chicks were infected by gently rubbing 0.05 cc. of the tissue suspension onto each of three sites, the head and comb, the left breast, and the right breast, previously prepared by plucking the feathers and superficially scarifying the skin.

Tissues for microscopic study were fixed in either Bouin's or Zenker's (5 per cent acetic acid) fluids, sectioned, and stained according to Giemsa's method, by means of hematoxylin and eosin, and by Masson's trichrome stain for connective tissue.

RESULTS

Pathogenicity for Chicks.—Our first attempt to produce lesions in chicks by employing a suspension derived from the tumor tissues of diseased grouse (fig. 4 and 5) indicated that we were dealing with an infectious agent closely related to the virus of fowl pox.

A single group of 24-hour-old chicks containing 4 birds was inoculated. The inoculum was prepared from the glycerolated tissues derived from the grouse killed in British Columbia, as described above. Within seven days, all of the birds had lesions at the sites of inoculation. These lesions were grossly (fig. 6) and microscopically (fig. 7, 8, and 9) indistinguishable from the lesions of fowl pox. Two of these birds were killed seven days after inoculation, one after twenty days, and the other bird was allowed to recover.

The results of this single experiment make it clear that the tumor tissue removed from grouse in nature contains an agent infectious for chickens. The application of a tissue suspension to previously scarified sites readily gave rise to a nodular cutaneous exanthem that rapidly enlarged to become confluent and wartlike in appearance.

In an attempt to learn whether the causal agent of the grouse disease can be maintained by serial passage in chicks, three experiments were carried out.

Three pairs of chicks, representing three successive experiments initiated at intervals of from seven to thirty days, were inoculated by employing tissue suspensions derived from tumors representing the preceding generation. Of these birds, all had typical bird pox by the seventh day after injection. One member of each pair was killed to provide material for microscopic

†It is a pleasure to thank Dr. Goodpasture for supplying us with known fowl-pox virus, and for reviewing histologic sections prepared from birds infected with the grouse strain of virus.

‡Alundum, an electrically fused crystalline alumina specially prepared for carbon determinations, was used because of its excellent "cutting" qualities.

LEGENDS FOR ILLUSTRATIONS ON OPPOSITE PAGE

Fig. 1—The head of one of the grouse that yielded tissue for the present study. (Fig. 1 is reproduced by permission of the editor of the Journal of Wildlife Management.)

Fig. 2—Cross section through the base of the lesion shown in fig. 1. Note the extensive proliferation of the epithelial cells, the superficial necrosis, and the sharp delimitation of the basal layer of epithelial cells from the underlying connective tissue. x30.

Fig. 3—A higher magnification of the growth of fig. 2. The epithelial cells are well-differentiated, but appear markedly abnormal in their internal structure. x150.

4

5

study and the other member of each pair was kept for immunity studies.

These three experiments show that the virus is readily transferable in series. Moreover, the short incubation period and the maximal and progressive reaction that characterized the induced growths from the first passage show that the agent recovered from the grouse is fully virulent for the domestic chicken. Accordingly, no further experiments were undertaken merely to transfer the causal agent in series.

Immunologic Studies.—The question next arose as to how the recovered birds would react to inoculation with known fowl-pox virus. The close resemblance of the newly induced disease to fowl pox, as it occurs either naturally or experimentally, suggested that chicks recovered from the disease under study might show some immunity to attempted infection with a virulent strain of fowl pox.

The site of initial inoculation and the normal skin of 4 chickens that represented four successive passages of the agent under study were tested from fifteen to forty-five days after injection for resistance to infection by the virus of fowl pox. None of these birds showed either a proliferative or other response which was in any way suggestive of the lesions of bird pox. On the other hand, 2 normal chickens that were inoculated for control purposes with the same suspension of fowl-pox virus developed typical fowl pox.

From the results of this experiment, it is concluded that chickens following recovery from an infection induced by the agent under study are solidly immune to infection by the virus of fowl pox.

Pathologic Anatomy.—It became apparent from the macroscopic appearance of successive changes in the induced lesions that we were dealing with an eruptive cutaneous growth essentially similar to bird pox. The application to prepared sites of a tissue extract of grouse origin was followed within seven days by smooth, circular and low convex epithelial papules that reached several millimeters in diameter and were confined to the epidermis. These papules rapidly enlarged to become confluent, rough, and wartlike cutaneous excrescences showing varying degrees of keratinization. Within ten to sixteen days after inoculation, the tumors frequently presented evidence of inflammatory change and regression. The inflammatory reaction was observed both superficially and at the base of lesions. The superficial type of reaction was present but rarely and probably resulted from the effects of secondary bacterial invaders. On the other hand, the inflammatory reaction at the base of the lesion occurred regularly as a natural regressive alteration. The other regressive changes consisted of desiccation, keratinization, and cleft formation with eventual formation of a dry scab. The scab sloughed to leave either a small amount of purulent material superficially or a smooth scar.

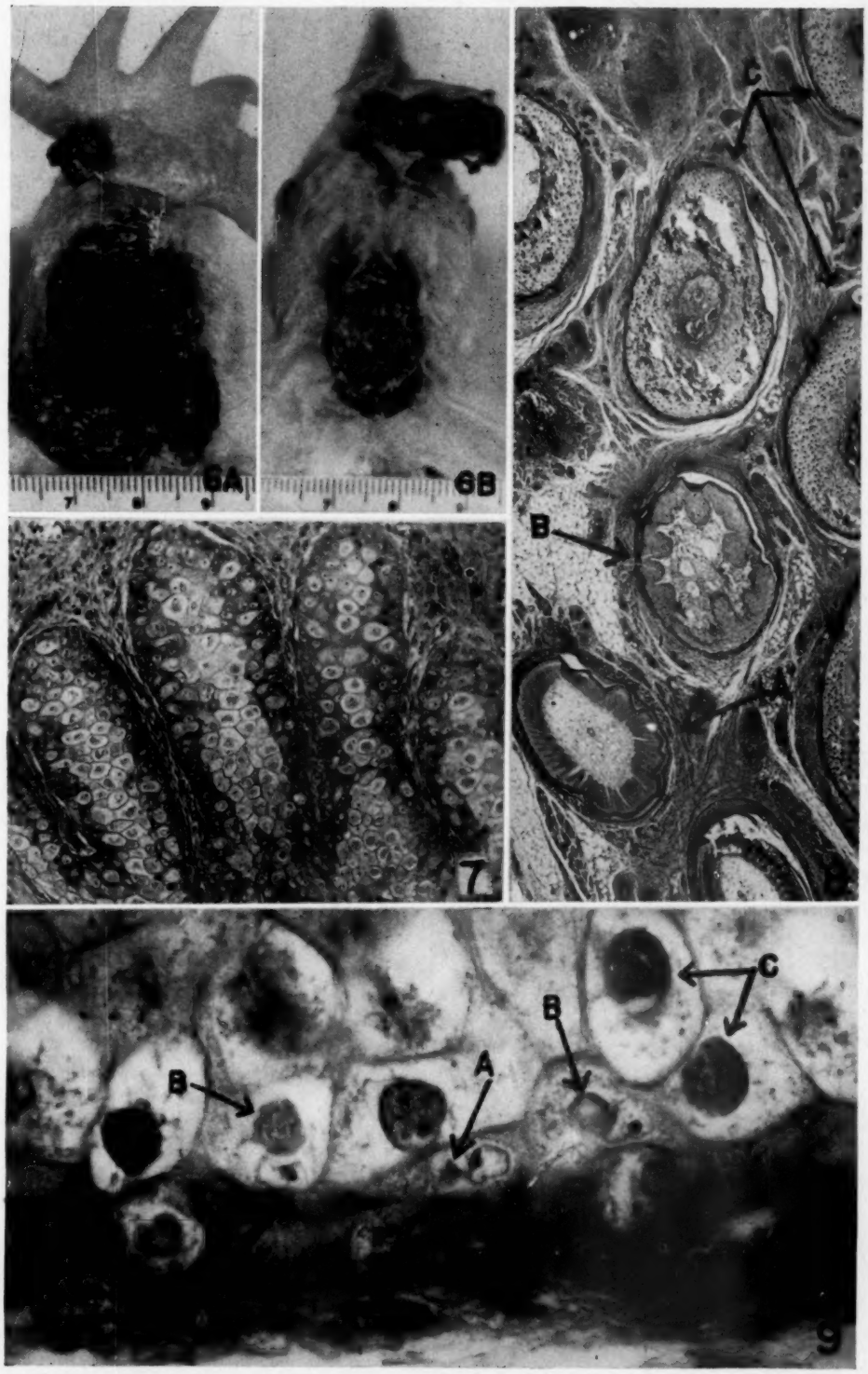
Sections were obtained from all of the chickens infected with the grouse virus. Histologic study of these sections showed that the induced lesions are entirely similar to the lesions of fowl pox (fig. 7, 8, and 9). The histologic changes are: proliferation of the epithelial cells in the stratum germinativum and the stratum granulosum of the skin (fig. 7) and feather follicles (fig. 8) with a resultant marked hyperplasia, and the formation of cytoplasmic inclusion bodies.

The most significant feature of the histopathologic picture is the presence of acidophilic cytoplasmic inclusion bodies (fig. 9). These inclusion bodies are found only in the epithelial cells at sites where

LEGENDS FOR ILLUSTRATIONS ON OPPOSITE PAGE

Fig. 4—Cross section of another lesion removed from a sooty grouse. The histologic picture is that of an infectious granuloma in which occasional nests of epithelial cells are embedded (arrow). x30.

Fig. 5—A higher magnification of the growth of fig. 4 to show one of the nests of epithelial cells. These cells are well-differentiated but in various stages of degeneration. A few of the cells contain Bollinger bodies. x150.


6A
6B
C
B
7
B
A
B
C
9

one can attribute the pathologic alterations to the specific infection. They are typically acidophilic and are variable in size, shape and structure. In their internal structure, they range from discrete granules to hyaline-like, homogeneous bodies that distend and distort the cell. The inclusion-bearing cells vary in size from normal to greatly hypertrophied cells. The increasing size of the inclusion body is accompanied by the ultimate destruction of the nucleus and the death of the cell.

The nature of the inclusion body was further investigated by employing a technique first described by Woodruff and Goodpasture.[9] The cytoplasmic inclusion bodies were freed from the surrounding host tissues by digesting these tissues with 1 per cent trypsin. The inclusion bodies were then ruptured by suspending them in sterile distilled water, allowed to dry on a slide, and stained by Giemsa's and Morosow's methods. Microscopic examination of the stained preparations revealed the presence of thousands of granules that approximated 250 μ in diameter. The nature of the inclusion body and its constituent elementary bodies helped prove that we were dealing with a virus of the bird-pox group, for the inclusion bodies were indistinguishable from Bollinger bodies and the elementary bodies from Borrel bodies.

DISCUSSION

The observation by one of us[8] of epithelial tumors on sooty grouse (*Dendragapus fuliginosus fuliginosus*) led to the present work in which a virus identical with fowl-pox virus is shown to be the causal agent of the malady. In all instances, the grouse strain of virus was readily passed to chicks and gave rise to exuberant lesions. There is no indication that this agent differs in its morphologic, pathogenic, and immunologic properties from a stock strain of fowl-pox virus. Previously, bird pox is not known to have occurred in grouse.

The natural host range of avian pox is limited to members of the bird family. Although the disease is reported to have occurred under natural or experimental conditions in a wide variety of birds,[2-4] more conclusive evidence[5] for its presence appears to be limited to the hen, *Gallus domesticus;* pigeon, *Columba livia;* turkey, *Meleagris gallopavo;* guinea fowl, *Numida meleagridis;* canary, *Serinus canaria;* partridge, *Perdrix cinereia;* quail, *Coturnix communis;* and pheasant, *Phasianus colchicus.* It will be noted that the nondomesticated birds in this group frequently are reared for restocking purposes near farms or human habitations where direct or indirect contact with barnyard fowl is possible at all times. It is not surprising, therefore, that outbreaks of bird pox occur occasionally when such susceptible birds as quail, pheasant, and partridge are exposed to the infectious agent. On the other hand,

LEGENDS FOR ILLUSTRATIONS ON OPPOSITE PAGE

Fig. 6-A—The head of a chicken twelve days after inoculation with the strain of virus isolated from sooty grouse. The tumors grew actively with marked proliferation of the epithelial cells and excessive keratinization.

Fig. 6-B—The head of a chicken twelve days after inoculation with a stock strain of fowl-pox virus for comparison with fig. 6-A. The only difference grossly is the increased size of the lesion induced by the grouse strain of virus.

Fig. 7—Cross section through the base of the growth in fig. 6-A. Note the columns of epithelial cells. x150.

Fig. 8—Cross section through feather follicles to show the alteration in appearance from infection of epithelial cells. The arrow A points to a normal feather follicle; arrow B to a follicle in which the epithelial cells are undergoing infection; and arrow C to follicles in which essentially all of the epithelial cells are infected. x150.

Fig. 9—A higher magnification of fig. 7 to show the development of inclusion bodies. Many of the cells of the stratum germinativum are normal in appearance. Arrow A points to a small Bollinger body in the cytoplasm of a cell in which the nucleus is unaltered; arrow B points to cells with well-informed Bollinger bodies in cells with intact nuclei; and arrow C to cells with large, hyalinized, acidophilic Bollinger bodies in cells in which the nuclei are not discernible.

it appears that the infection of wild birds by avian-pox virus occurs but rarely since an overt infectious malady, manifest by nodular and wartlike cutaneous lesions and frequently by a high mortality, is not likely to escape detection for long. The discovery of avian pox as a natural infection in sooty grouse is of interest, therefore, as it gives evidence for an increased host range and economic importance of the disease.

An epidemiologic implication of these findings is worthy of consideration. Although the sooty grouse is limited in distribution to the Pacific Coast region, related genera and species are widely distributed throughout North America. The presence of fowl pox as an epiornithic infectious disease of sooty grouse for several seasons suggests, therefore, that this infection may appear with serious consequence among other wild fowl. The gregariousness under natural conditions of many of the game birds favors the transmission and maintenance of this disease.

Whenever a naturally occurring outbreak of an infectious disease of birds is characterized by proliferation of cutaneous epithelium with the formation of wartlike nodules that are situated principally on the unfeathered parts of the fowl, it is probable that the malady is a variety of bird pox. The predominantly proliferative nature of the lesions with only a superficial resemblance to the exanthematous pox diseases of mammals frequently leads to the mistaken impression that the condition is cancer. The accompanying granulomatous changes are attributed to chronic inflammation. It is, of course, because of this that Bollinger[1] originally classified the lesion with tumors and gave it the name "epithelioma contagiosum," a synonym unfortunately employed for its designation to this day.

It apparently is not clear whether fowl pox and avian diphtheria constitute a single entity or two distinct diseases.[6,7] This confusion has arisen because in epiornithic outbreaks among fowl the cutaneous manifestations characteristic of fowl pox are frequently associated with an exudative reaction involving the mucous membranes of the eyes, nose, mouth, throat, and trachea. An infection limited to the mucous membranes is diagnosed clinically as avian diphtheria. As both virus isolated from a natural outbreak and passage virus were employed in the present study, it is worthy of mention that we did not observe catarrhal or caseous exudates, or pseudomembranous patches, involving either the conjunctivae or the mucous membranes of the respiratory or gastrointestinal tracts in either the experimental or captured wild birds.

SUMMARY

The virus of fowl pox (contagious epithelioma of fowl) was the causal agent of a naturally occurring infectious disease of sooty grouse (*Dendragapus fuliginosus fuliginosus*) in the Cowichan Valley of southeastern Vancouver Island, British Columbia. The affected tissues of the diseased grouse readily yielded a virus that is indistinguishable from a known strain of fowl pox in its pathogenic, histopathologic, and morphologic characteristics. Moreover, chickens recovered from an infection with the causative agent of the grouse disease are solidly immune to attempted infection with a massive dose of fowl-pox virus. This is the first time that the sooty grouse has been implicated as a natural host for the virus disease, fowl pox.

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The Uterine Wall of the Cow

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FOR THOSE who can interpret the signs, there is written in the uterine wall the story of how the sperm, ovum, embryo, and fetus are accommodated, conserved, nourished, and born. There is also recorded the causes of failure and death of these bodies. The characters in which this story is written are not letters, words, and phrases, but cells, fluids, tissues, and reactions. Some of the signs are macroscopic and clinical, such as redness, heat, pain, and swelling. Others are microscopic, such as changes in epithelial cells, connective tissue cells, and leucocytes and glands.

The objective herein is: (1) to discuss the physiologic phenomena that are prerequisite to an understanding of those changes that take place in the uterine wall during quiescence and estrum, and in preparation for processes leading up to gestation and parturition; (2) a study of the architecture of the uterine wall with particular emphasis upon those fluids, cells, and tissues which achieve: (a) the prevention of invasion of microörganisms, and (b) the repair of injury to the uterine wall (chart 1).

The cross section of the uterine wall of the cow shows about the same construction that is found in other species, that is, first, the endometrium; second, the vascular bed; third, the muscles; and fourth, the serous coat. The subdivisions of the endometrium are as follows: epithelial layer, 1.1; lamina propria, 1.2; compacta, 1.3; and spongiosa, 1.4; making up what is called the functionalis, and on the bottom of the endometrium lies the basalis, 1.5. This establishes a routine procedure that conforms with studies conducted upon other species. Beneath the endometrium and between the circular and longitudinal muscle layers is the vascular bed (layer 3, chart 1).

The incentive for studying the uterine wall is stimulated by the importance of its functions, and its exceptionally versatile properties.

It forms the tube and secretes the mucus that aids in the transportation of the sperm to the ovum and the return trip of the fertilized ovum to the uterine horn. It develops a matrix of itself for the nesting of the fertilized ovum. It provides nutrition and respiration for the developing fetus. It governs the length of time the fetus can remain inside the mother. It receives and reacts to endocrine and nervous messages from the brain and ovary. It is, therefore, one link in the endocrine chain. It takes part in the storage, and perhaps the manufacture, of endocrines. Its handling of fluids—blood and lymph—is miraculous. It possesses a reparative system that makes temporary and permanent repairs, and later erases much of the evidence of the damage that has been sustained by injurious agents.

An understanding of the uterine wall is prerequisite to the solution of such problems as abortion, retained fetal membranes, and infertility. Each is a definite challenge to the clinician. In spite of the fact that these problems have been studied and treated and handled for years, little is actually known about them.

Following the example set by mathematicians, chemists, and other scientists, who depend upon fundamental formulas for the solution of specific problems, the gynecologist has at hand an endocrine-relation system that presents a formula for the solution of certain kinds of infertility. Chart 2 is a diagrammatic representation of an endocrine formula. It shows the course of some of the most important endocrine products, especially those responsible for estrum, nidation, placentation, and nutrition of the fetus. Much of our knowledge of endocrine influence upon the genital tract is derived from small animal experimentation and from studies of the genital tract of the

human subject. It is essential that we understand and take advantage of those fundamental steps. However, the species difference makes the task of correlation a difficult one, and one must not attempt to make literal comparisons since the human uterus is decidual and the bovine adecidual. Let us study some of these endocrine influences in order to refresh our memories and make the rest of the paper more understandable. The pituitary gland secretes a follicle stimulating hormone and a lutein stimulating hormone. The physiologic mature ovary accepts this endocrine stimulation and in its turn secretes hormones that act upon the uterus. The first of these is produced by the follicle and goes by a number of names, estrin, folliculin, theelin, and synthetic diethylstilbestrol. The second hormone comes from the corpus luteum and is called progesterone. The response of the uterus to these two hormones can be divided into phases or stages called the follicular and luteal reactions.

A marked differentiation between the histologic changes produced by estrogenic substances and the phenomena that result from the luteal stimulation have not been, and perhaps cannot be, clearly defined in the endometrium of the cow. I think that we should not expect a well-defined differentiation in an adecidual uterus. Certainly, we should not expect to find the proliferative thickening of the endometrium as it occurs in the human subject, later to be desquamated. True menstruation does not take place in the cow. The estrogenic phase has no definite limitations. It is characterized by a vascularization and edema of the endometrium, and this acute reaction or congestion merges into what we may call the luteal or progesteronic phase where the glands become more tortuous and the area more congested and the cytoplasm of the glands higher. At this time, the cytoplasm throws out threadlike projections into the lumen of the gland. The gland, therefore, takes on what may be termed a se-

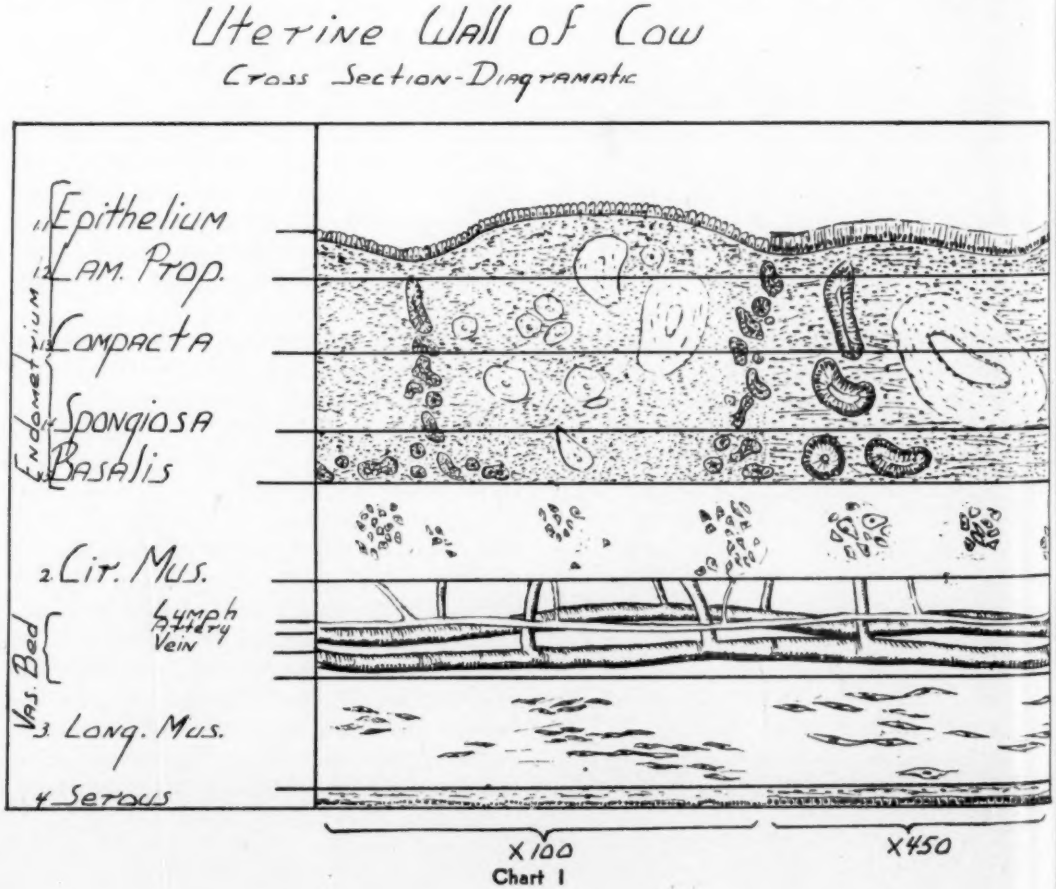

Chart 1

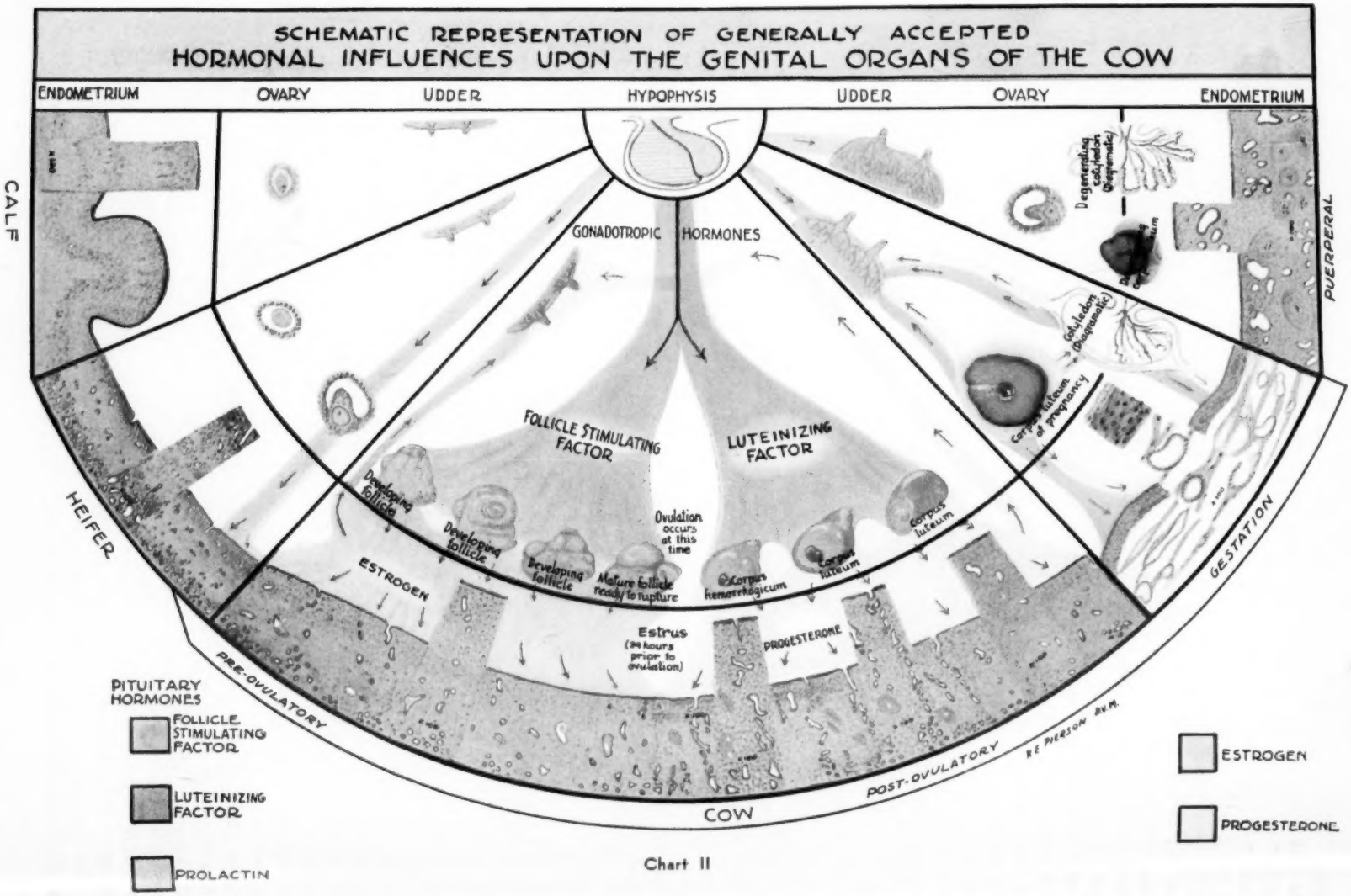
SCHEMATIC REPRESENTATION OF GENERALLY ACCEPTED
HORMONAL INFLUENCES UPON THE GENITAL ORGANS OF THE COW
ENDOMETRIUM
OVARY
UDDER
HYPOPHYSIS
UDDER
OVARY
ENDOMETRIUM
CALF
HEIFER
PUERPERAL
GESTATION
GONADOTROPIC HORMONES
FOLLICLE STIMULATING FACTOR
LUTEINIZING FACTOR
Developing follicle
Developing follicle
Developing follicle
Mature follicle ready to rupture
Ovulation occurs at this time
Corpus hemorrhagicum
Corpus luteum
Corpus luteum
Corpus luteum of pregnancy
Degenerating cotyledon (Degenerative)
Cotyledon (Progressive)
ESTROGEN
Estrus (24 hours prior to ovulation)
PROGESTERONE
PRE-OVULATORY
POST-OVULATORY
COW
R.E. PIERSON A.V.M.
PITUITARY HORMONES
FOLLICLE STIMULATING FACTOR
LUTEINIZING FACTOR
PROLACTIN
ESTROGEN
PROGESTERONE

Chart II

cretory phase. If gestation ensues and the fetal membranes become attached to the endometrium, the situation is an entirely different one from that encountered in the types of uteri in which a diffuse attachment takes place. In the case of the cow, the attachment takes place in those areas which we call caruncular areas which consist of blood vessels terminating at the apex of a small rounded elevation. This later develops into a complicated organ known as a cotyledon and a placenta. During gestation, the epithelium of the intercotyledonous area becomes diminutive in height, the nucleus is small, and there is a single layer of cells instead of the piling-up of cells that is found in the nongravid uterus. The glands themselves become greatly distended and they too are lined by a single layer of cells. There is no evidence of a secretion of mucus at this time. The lamina propria becomes thin, as does the rest of the uterine wall.

For the moment, I should like to return to a study of the surface of the uterus and a discussion of the mucus in which it is bathed. The defense of the uterine wall against bacterial invaders and the repair of the tissues following injury begins with mucus. This is true of all mucous membranes, but in no instance does it accomplish more phenomenal results than in the case of the uterus. Immediately following attack by an irritant, mucus is produced in large amounts. At first, it is thin and watery. It mechanically removes bacteria by lavage of the surface. Then it becomes thicker and viscous, immobilizing organisms, covering the epithelial layer with a gelatinous coat, thereby giving the epithelial cells a chance to multiply and perform their reparative duties. The mucus becomes a medium for sera, and a vehicle in which leucocytes attack and finally a means for carrying bacteria and débris from the body. Mucus is of great clinical significance since its physical properties are characteristic of the state of health or disease of the tissues that lie beneath it. It tells the story of the kind, degree, and time that certain reactions in the uterine wall have occurred, and whether these reactions have been physiologic or pathologic. Therefore, it becomes important, though sometimes difficult, to differentiate between physiologic and pathologic phenomena. It is not the intention to deal with the character of uterine mucus under all conditions, but to call attention to the fact that it is a part of the uterine wall and that, perhaps, it is of greater clinical value than all the other factors taken together.

I am led to believe that uterine mucus always harbors macerating epithelial cells. At all times, there is just enough mucus to moisten the surface and make it glisten. Mucus in itself is moderately bacteriocidal and it provides a medium where the phagocytic agents can meet and conquer invading organisms. Turbid mucus is not pathognomonic of endometritis.

Up to this time, we have been describing the uterus and not including the cervix and its secretions. The cervix remains closed by mucus even during estrum and although the uterus is flushed by increased granular activity, I doubt whether any of the fluid of the nongravid uterus escapes through the cervix. Following parturition, the surface is thoroughly bathed and cleansed by the secretion of mucus that carries with it disintegrating blood clots and membranous débris. It terminates by a flow of chocolate colored or yellow mucus from the puerperal uterus, and is termed lochia. It embodies nature's most efficient method of cleansing and repairing the changes sustained by the uterus during gestation. Therefore, for the fear of doing more harm than good, one should hesitate about interfering with this natural defensive factor and be very cautious about the use of antiseptics or other so-called remedial agents.

Immediately beneath the thin coat of mucus lies the epithelium, the defensive properties of which are phenomenal. We are familiar with this protective sheet of epithelial cells and its effectiveness as a barrier to invasion in many parts of the body. The part it plays in the uterine wall is no exception. Old cells are being constantly replaced by new ones. They con-

tinually change in shape and size under the influence of estrin and progesterone. Epithelium can perform its multitudinous duties only in the presence of an adequate provision of vitamins A, C, and E, and perhaps others.

Beneath the epithelium, the connective tissue cells of the basement membrane and the lamina propria take over the secondary defense. I can think of no place in the body in which the defense is more effective and the repair more complete than in the case of the lamina propria. The density of connective tissue cells is greatest in the area immediately beneath the epithelium. Beneath the lamina propria, the connective tissue cells are less numerous and still deeper in the "functionalis" they permit the accumulation of fluid and allow space for cyclic changes in the glands. This zone is so loose in comparison to the superficial layers that it is called spongiosa. It forms a bed of connective tissue cells and intercellular substance, collagen, the integrity of which is dependent upon the presence of adequate amounts of vitamin C. It is evident that any deficiency in health of the spongiosa would be reflected in the vitality of other tissues, especially the epithelium. Macroscopically and microscopically, a strenuous effort is being made to examine the "functionalis" and to interpret the picture it presents. The cyclic changes in the uterine wall of the human uterus have been studied thoroughly, and there is a uniformity of opinion among pathologists in regard to the microscopic pictures and the endocrine secretions that produce them. Gynecologists make valuable use of the microscopic picture of the human endometrium, and biopsy is a routine measure.

The hormones produced by the human subject and by lower animals are the same, but the responses on the part of the uterine wall are comparable but not parallel. Any effort to make direct analogies is doomed to disappointment. Unfortunately, the veterinarian is denied access to many of the measures that are routine procedures in human practice. The problems confronting the veterinarian are difficult and progress is slow, but we propose to improve our position by hard work and by taking advantage of the contributions made in the fields of experimentation and texts written for the human subject. Just how much attention the clinicians should give to the microscopic picture may be controversial. It is admitted that it is not essential that one should know the details of one's watch and all the intricate mechanisms and correlations of wheels and bearings in order to tell the time of day, but the man who repairs the watch must know. This is the difference between the clinician and the layman, except that the uterine wall is much more difficult to understand than the most delicate timepiece.

The uterus adapts itself quickly to the changes of pregnancy; it survives the trials of parturition, and repairs the damage of separation of fetal membranes and the injury that comes from bacterial or traumatic insult. The connective tissue as well as the epithelial defense of the uterine wall is complemented by a leucocytic invasion of mobile cells that wander about the lymph spaces and take care of invaders that may have penetrated epithelial and connective tissue barriers.

The defense of the uterine wall can not be discussed without appreciation of the part played by the vascular bed. It seems to me that this has been neglected by our pathologists. However, clinicians have learned much about its significance. In addition to handling the flow of blood and lymph confined to vessel walls, it forms a veritable reservoir for extravascular fluids—sometimes physiologic and sometimes pathologic. An indication of the load that the vascular bed is carrying is reflected in the external genitalia since the vulva and vagina also serve as reservoirs for the overflow of fluids that can not be stored, even temporarily, in the vascular bed of the uterine wall.

The clinician differentiates very carefully between the edema of approaching parturition and the edema of fulminating septicemia. An error in judgment here means the difference between life and death.

Further discussion of those delicate problems must be delegated to the chapter upon diseases of the puerperal uterus. The muscles play an important rôle in the defense of the uterine wall. It is the well-known strategic move on the part of the militarists to shorten their lines. In the case of the uterine wall, this defensive strategy is accomplished by the muscular layers. The muscle reduces the vulnerable surface of the uterine wall from feet to inches. It expresses excessive fluids from the lumen of the uterus and performs many functions that we have not space to discuss. It is directly under the stimulating influence of the estrogens and pituitrin and the inhibitory actions of progesterone.

The serous coat is the last wall of defense and performs its duties in much the same way as the epithelial layer.

It must not be forgotten that the endocrine influences are also defensive in their action. This is particularly true of the estrogenic substances. They stimulate the glands into action and constitute the principal factor in bringing about muscular contraction. Their therapeutic use is not thoroughly appreciated by the veterinary profession. We are not familiar with its value in the uterine inertia, or just when and how to use it. I suspect that ingestion of fetal membranes by the cow may be beneficial rather than harmful because of the estrogen and possibly the vitamin C content of those membranes. Estrogenic products have been used successfully in the evacuation of the uterus in missed abortions.

The excessive action of estrogens causes endometrial glandular hyperplasia as is seen in the case of nymphomania. It is of intense interest that the reaction on the part of the uterus to estrin, and perhaps progesterone, resembles the initial phases of inflammation: congestion, leucocytic infiltration, diapedesis, and edema, followed by repair. It requires no stretch of the imagination to suspect that estrin and progesterone are proving to be valuable therapeutic agents. The gynecologist in human practice is finding uses that probably will not apply to lower animals, but from the standpoint of promoting the defensive and reparatory powers of the uterus, we can have great hopes.

Based upon its physiologic action, the estrin group of therapeutic agents will be of value in certain kinds of endometritis, retained placenta, and endometrial hypoplasia. Its usefulness will grow as the clinician becomes more and more familiar with the things that take place in the uterine wall.

Estrin is on the market in the form of theelin, folliculin, stilbestrol, and many other agents. We know little of its indications and dosage.

This paper deals briefly with a number of problems relating to reproduction in the cow. It makes no pretense to finality. I feel that progress is being made and that sufficient work has been done to warrant reporting. It is hoped that it will stimulate discussion and a greater effort on the part of all of us.

The Effectiveness of a 1-to-14 Phenothiazine-Salt Mixture in Controlling Nematode Infection in Sheep

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EVER SINCE the epochal discovery in 1939 of the anthelmintic properties of phenothiazine, attempts have been made to simplify the methods of administering the drug to the various animals, especially sheep. One of the more recently suggested methods is the incorporation of the material in salt licks. Habermann and Shorb[1] found phenothiazine-salt mixtures in proportions of from 1:9 to 1:14 to be effective in reducing the larval counts in fecal cultures. The average daily consumption of phenothiazine was at least 0.5 Gm. per sheep. Earlier, Shorb and Habermann[2] pointed out that daily administration of 0.5-Gm. doses of the drug completely inhibited the development of all nematode larvae with the exception of *Strongyloides papillosus*. Viable larvae again appeared in the fecal cultures forty-eight hours after withdrawal of the drug. The egg count was not at any time reduced. Gordon[3] arrived at essentially the same conclusions. Britton, Miller, and Cameron,[4] in an uncontrolled observation with 830 ewes and lambs, reported a definite anthelmintic effect of a 1:15 phenothiazine-salt mixture. In a second and more comprehensive report involving controlled experiments, the same authors[5] again report favorably upon a 1:15 phenothiazine-salt mixture as a practical and effective anthelmintic for sheep. A 1:9 phenothiazine-salt mixture, according to Thorp and Keith,[6] reduced the numbers of nematode ova in the feces of parasitized sheep. The elimination of ova remained low as long as the mixture was fed. Foster and Habermann[7] recommend the use of mixtures containing from 9 to 14 parts of salt to one part of phenothiazine in the control of ovine nematodes. They caution, however, that the use of phenothiazine in salt should be regarded as a preventive rather than a therapeutic procedure.

Thorning, Sampson, and Graham[8] presented evidence to suggest that phenothiazine in appropriate doses is quite effective if administered to sheep in a capsule, in a bolus, as a drench, or incorporated in soybean pellets. The present work has been designed to obtain further information on the effectiveness of a phenothiazine-salt mixture in controlling internal parasitism in sheep.

PLAN

Approximately 600 mature western ewes, maintained at the Dixon Springs Agricultural Experiment Station in Pope County, southern Illinois, were made available for the experiment. Early in January, 1943, the sheep were brought in from pasture for drylot feeding and divided at random into 3 lots of 196 ewes each. Each ewe was dosed with 2 oz. of a 50 per cent suspension of phenothiazine in molasses and water. They had been dosed similarly in August, September, October, and November of the previous year. Mixed hay (mainly lespedeza and grass) was fed to all lots, together with 1 lb. shelled corn and 0.25 lb. of soybean oil meal per head daily. Beginning Jan. 15, 1943, a 1:14 phenothiazine-salt mixture, free choice, was made available to the ewes in lot 3. In addition, the ewes in lot 3 were given access to a separate mixture of limestone and bonemeal without salt. Lots 1 and 2 each had access to a single mixture of salt, limestone, and bonemeal, free choice.

Lambing occurred in the 3 lots during February, March, and April. The experimental treatment of these lots of ewes and lambs was planned as follows: Lot 1, the control group, was to be maintained without further phenothiazine medication. Lot 2 was to be dosed with phenothiazine (1 oz. per ewe, 0.5 oz. per lamb) at the beginning of the pasture season and thereafter maintained on a 1:14 phenothiazine-salt mixture. Lot 3 was to be maintained continuously both in the drylot and at pasture on the 1:14 phenothiazine-salt mix-

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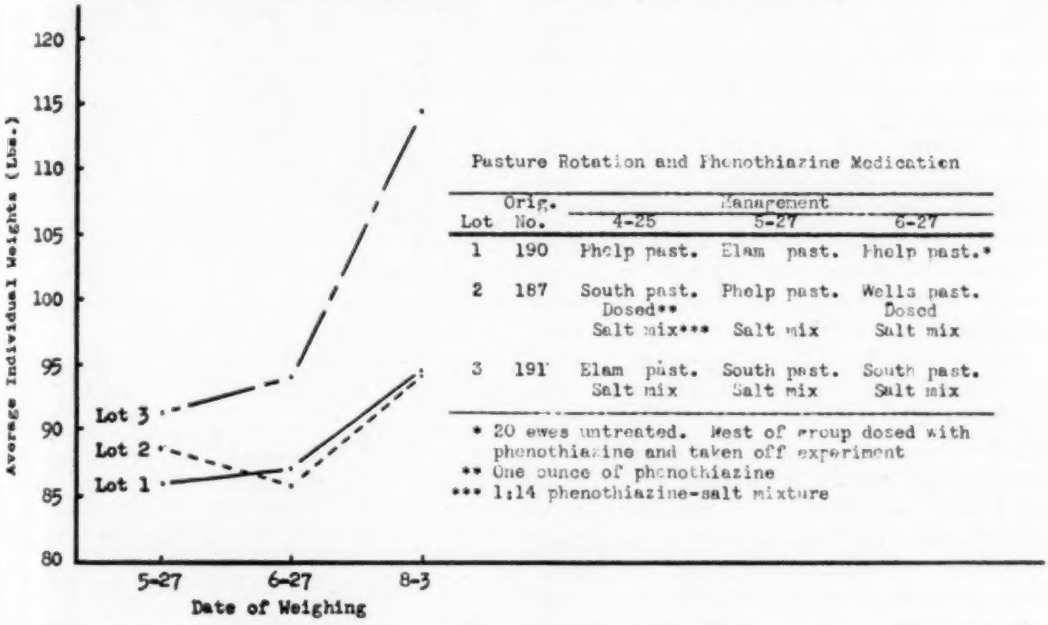
ture. In order to limit losses, however, it was planned to treat the sheep in any lot when and if parasitism became marked.

The degree of parasitism was to be estimated by fecal examinations for the presence of nematode ova and by autopsy examinations of representative ewes and lambs at time of marketing. The rate of gain and general condition were to be determined by inspection and periodic weighing.

The pastures ranged from 100 to 300 acres in size. Because of an unusual amount of rain during the spring and early summer, growth of forage was excellent and ample. With one exception, the pasture for each lot was changed once a month.



Pasture Rotation and Phenothiazine Medication

Lot	Orig. No.	Management 4-25	5-27	8-27
1	190	Phelp past.	Elam past.	Phelp past.*
2	187	South past. Dosed** Salt mix***	Phelp past. Salt mix	Wells past. Dosed Salt mix
3	191	Elam past. Salt mix	South past. Salt mix	South past. Salt mix

* 20 ewes untreated. Rest of group dosed with phenothiazine and taken off experiment
** One ounce of phenothiazine
*** 1:14 phenothiazine-salt mixture

Fig. 1—Record of Ewes.

FECAL EXAMINATIONS AND POSTMORTEM INSPECTION

At the beginning of the pasture season and continuing at monthly intervals throughout the spring and summer, fecal specimens were collected from a random selection of 25 ewes and 25 lambs in each lot. A similar collection had been made from the ewes in lot 3 early in January. When preliminary observations (flotation) showed nematode ova to be present, a 2-Gm. portion of each fecal sample was preserved with 2 cc. of 25 per cent formalin. The specimens were then returned to the Department of Animal Pathology Laboratory at Urbana for determination of the nematode ova present according to the Stoll technique. At the time of slaughter, the intestines from representative animals were observed for the presence and the relative numbers of parasitic nodules. The abomasum was also opened and a portion of the contents removed and preserved in formalin for nematode examination.

RESULTS

Clinical Observations and Relative Gains.—The ewes, together with their lambs, were turned to pasture April 25. The system of pasture rotation is set forth in table 1.

During the first month on pasture, none of the sheep developed clinical manifestations of parasitism. It was expected, however, that the pasture occupied by the sheep in lot 1, the untreated group, would become contaminated and that this pasture, subsequently occupied by the second lot, would serve to test the effectiveness of a 1:14 phenothiazine-salt mixture in protecting ewes and lambs from parasites when ex-

posed to infection. Lot 3, the lot maintained continuously upon phenothiazine-salt mixture since January, was never exposed to similar infection during the current season.

At the end of the second month at pasture, the ewes and lambs in lot 2, given access to a 1:14 phenothiazine-salt mixture, but maintained for four weeks upon a contaminated pasture, had developed marked clinical manifestations of parasitism. The sacrifice of 2 ewes and 1 lamb from this group confirmed the diagnosis. All ewes and lambs in this lot were treated with phenothiazine and placed on clean pasture. Both ewes and lambs in lot 1 also showed evidences of parasitism, but not to the same degree as those in the second group. A remnant of this lot, consisting of 20 ewes with their lambs, was maintained without treatment. The rest of the control group was dosed with phenothiazine and placed on clean pasture, thereby removing them from the experiment. Their subsequent history will not be followed in this report. The ewes and lambs in lot 3, maintained upon a 1:14 phenothiazine-salt mixture but not placed upon a pasture recently occupied by heavily parasitized, untreated sheep, remained in good condition. Their pasture was not changed. Weight variations and pasture management of the respective lots are included in figures 1 and 2.

TABLE 1—Pasture Rotation Among the Three Lots of Ewes and Lambs

Time at Pasture	Pasture		
	Elam	South Phelps	Wells
April 25—May 26 ...	Lot 3	Lot 2	Lot 1
May 27—June 28	Lot 1	Lot 3	Lot 2
June 29—August 3 ..		Lot 3	Lot 1* Lot 2

*20 ewes and 20 lambs of lot 1 were maintained without treatment. The remaining animals of this group were dosed with phenothiazine and taken off the experiment.

The total gains registered by lots 2 and 3 during the interval in which weights were recorded are given below in table 2.

Inspection of figures 1 and 2, and table 2 indicates that the sheep in lot 3, maintained continuously upon a 1:14 phenothiazine-salt mixture but not placed on pasture recently occupied by heavily parasitized, untreated sheep, made satisfactory gains. The second lot, given the same phenothiazine-salt mixture but turned upon a contaminated pasture, became badly damaged in one month. Even though treated individually with phenothiazine and placed on clean pasture while still being maintained on the 1:14 phenothiazine-salt mixture, the 350 ewes and lambs originally in this lot made essentially no gain during the ten-week interval in which weights were recorded. The 348 ewes and lambs in lot 3 during the same period made a total gain of 8,670 lb. The control group (lot 1) made poor gains.

Egg Counts

In table 3, a summary is given of the nematode ova contents of the fecal specimens as determined by the Stoll technique. As stated before, 25 fecal samples were collected at monthly intervals from the ewes and from the lambs in each lot. The results given in table 3 are the logarithmic averages (nematode ova per gram of feces) of each series of 25 samples.

TABLE 2—Total Gains Made by the Ewes and Lambs in Lots 2 and 3 from May 27 to August 3 (Ten Weeks)

Lot		Original Number	Mortality	Total Gain
2	Ewes	187	4* (2.1%)	710 lb.
2	Lambs	163	13* (8.0%)	—700 lb.
		350	17 (5.0%)	10 lb.
3	Ewes	191	1 (0.5%)	4,270 lb.
3	Lambs	157	0	4,400 lb.
		348	1 (0.3%)	8,670 lb.

*Two ewes and one lamb sacrificed for autopsy.

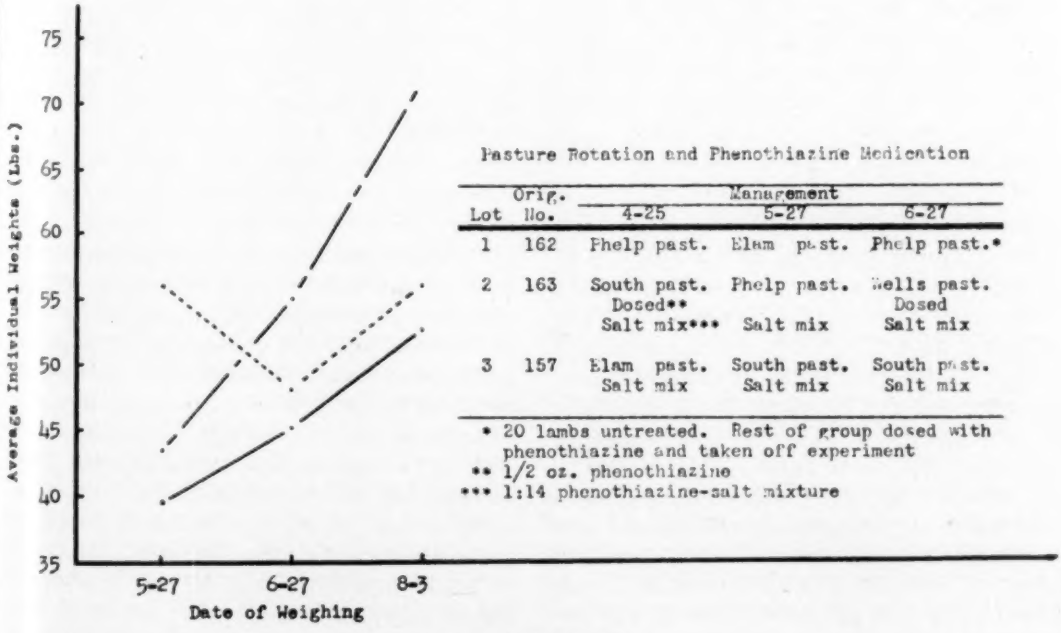
The nematode ova determinations of fecal samples as a means of estimating the degree of parasitism and as a measure of anthelmintic efficiency proved in this experiment to be of limited value. It would be deduced from table 3, for example, that the ewes in lots 1 and 2 initially were heavily parasitized at the time they were turned to pasture, but subsequently eliminated most of the infection. It would be further deduced that the lambs in lot 1 were more heavily parasitized than those in lot 2. These deductions are not supported by observations on comparative rates of

gain, mortality figures, and postmortem examinations. Just why nematode ova determinations of fecal specimens according to the Stoll technique failed to correlate with clinical manifestations is not clear, but probably several factors are involved. One of these would be variations in fecal moisture content. The feces of sheep on drylot contain less water than when on pasture, and the feces of healthy sheep are more solid than those of parasitized animals, the latter often showing profuse diarrhea. An increase in the fecal water content obviously would reduce the ova obtained per unit mass of feces in parasitized sheep. Leiper and Peters[9] determined statistically that variations in weight are more sensitive indicators of anthelmintic efficiency than are egg counts. Our observations tend to support this conclusion.

Consumption of the Phenothiazine-Salt Mixture.—During the period of observation, 1,173 lb. of the 1:14 phenothiazine-salt mixture were fed to the ewes and lambs in lots 2 and 3. This represents a monthly consumption of 55 lb. per 100 head of sheep, or a phenothiazine consumption of 3.7 lb. On an individual basis, the average daily consumption of the drug per animal was 0.55 Gm. Based on the current price of phenothiazine in 100-lb. lots, the cost of the drug consumed by each sheep was 2.5 cents per month. For smaller orders of phenothiazine, the cost of medication would be somewhat greater.

Postmortem Examination.—Portions of the intestinal tracts of 133 lambs from the three lots and the 20 untreated ewes in lot 1 were examined at the time of slaughter. The intestine was examined for the presence and the relative numbers of parasitic nodules while the abomasum was opened and inspected for the presence of the stomach worm. All examinations were necessarily qualitative in nature due to the impossibility of slowing up plant operations. A summary of these results is given in table 4.

It is seen from the foregoing table that the lambs in lot 3, even though making satisfactory progress, nevertheless, suffered from a degree of parasitic infection. From the large numbers of nodules found on the intestines, the lambs in lot 2 evidently were the most heavily parasitized of the groups examined. This group made the poorest



Pasture Rotation and Phenothiazine Medication

Lot	Orig. No.	Management 4-25	5-27	6-27
1	162	Phelp past.	Elam past.	Phelp past.*
2	163	South past. Dosed** Salt mix***	Phelp past. Salt mix	Wells past. Dosed Salt mix
3	157	Elam past. Salt mix	South past. Salt mix	South past. Salt mix

* 20 lambs untreated. Rest of group dosed with phenothiazine and taken off experiment
** 1/2 oz. phenothiazine
*** 1:14 phenothiazine-salt mixture

Fig. 2—Record of Lambs.

gains and suffered the greatest mortality (fig. 2, table 2).

The ewes and lambs in the control group (lot 1), as would be expected, showed marked parasitism. It is of interest to note, however, that this group, although untreated since January, suffered less damage from parasitism than the treated ewes

TABLE 4—Postmortem Examination of 153 Sheep

Lot		No. Examined	Stomach Worm Infection	Nodulation on Intestines		
				Free	Moderate	Heavy
1	Ewes (untreated)	20	Moderate	0*	4*	16*
1	Lambs (untreated)	18	Heavy	0	16	2
2	Lambs	20	Light*	0	0	20
3	Lambs	95	Light	6	83	6

*The ewes undoubtedly had nodules persisting from the previous year, while the lambs in lot 2 had been treated individually with phenothiazine a month previously.

and lambs in lot 2. This apparent inconsistency is explained by the system of pasture rotation used (table 1). It may reasonably be assumed that the exposure to infection in lot 2, one of the groups upon a 1:14 phenothiazine-salt mixture, was greater than that in lot 1. Lot 2 suffered the greater damage.

As previously mentioned, all ewes placed on this experiment had been dosed individually in August, September, October, November, and January with 1 oz. of phenothiazine. A residue of infection survived even this vigorous treatment and at once became active in unprotected sheep upon summer pastures.

Discussion

It is known that the administration of small, repeated doses of phenothiazine to sheep such as would be received in a salt mixture, probably exerts its major anthelmintic action not through elimination of worms from the intestinal tract, but from a suppressed development of the eggs to the infective larval form. This is clearly indicated in the observations of Shorb and Habermann,[2] Habermann and Shorb,[1] and Gordon.[3] It appears, therefore, that the greatest benefits should be derived from the feeding of a phenothiazine-salt mixture when sheep, relatively free from internal parasites, are placed upon an uncontaminated pasture.

Such a plan for nematode control in sheep is made more practical by recent disclosures that the larvae of these parasites do not survive as long on pastures as has previously been thought. As a result of limited experiments, Kammlade, Graham, and Boley[10] made such a suggestion as far back as 1936. More recently, Sarles[11,12] and Kates,[13] all working in the vicinity of Washington, D. C., have shown that pastures, heavily contaminated with egg-containing feces during one grazing season, became largely noninfective for parasite-free lambs the following spring and summer. In an enlightening article, Dinaburg[14] demonstrated that under the conditions of his experiment, there was near if not complete destruction of *Haemonchus contortus* larvae

TABLE 3—Logarithmic Averages of Nematode Ova Determinations

Lot		Date of Collection				
		1—15	4—27	5—27	6—28	8—3
1	Ewes		4,600	225	180	190
2	Ewes		2,800	52	20	24
3	Ewes	4	165	91	32	11
1	Lambs			37	3,300	3,900
2	Lambs			22	1,036	274
3	Lambs				793	48

after 42, 83, 98, and 316 days in the summer, winter, spring, and fall experiments, respectively. Using laboratory procedures only and working with *H. contortus*, Shorb[15] failed to demonstrate over-winter survival of the larvae.

In an earlier and even more revealing article, Shorb[16] showed the survival period of the common nematode larvae, parasitic for sheep, to be limited on summer pastures even in the presence of abundant shade and moisture. One such contaminated pasture apparently became noninfective for parasite-free lambs in the short space of only two months. It is shown in the same paper that eggs passed during the winter and early spring do not develop to the infective larval stage. Feedlot infection of sheep, therefore, except during periods of unusually warm weather, may be of negligible importance.

From the observations reported in this

paper and the other papers mentioned, a practical procedure for nematode control in sheep for the northern states might tentatively consist of individual, prepasture treatment of the ewes with the therapeutic dose of phenothiazine and their maintenance thereafter at pasture, together with the lambs, on a phenothiazine-salt mixture. Further observations may be necessary to determine whether or not the sheep should again be treated individually with phenothiazine at the end of the pasture season. For best results, the animals should not be maintained at any time upon pasture occupied by parasitized, untreated sheep during the preceding two months.

SUMMARY AND CONCLUSIONS

A lot of 348 sheep (191 ewes, 157 lambs), maintained continuously upon a 1:14 phenothiazine-salt mixture throughout the drylot and grazing seasons and handled as an isolated unit without contact, direct or indirect, with heavily parasitized, untreated sheep, made a total gain of 8,670 lb. from May 27 to August 3 (ten weeks). One animal died. Their progress was considered satisfactory. Postmortem examinations of 95 lambs from this group showed internal parasites to be present, nevertheless. The phenothiazine-salt medication, therefore, markedly suppressed but did not eliminate nematode infection.

A second lot of 350 sheep (187 ewes, 163 lambs), treated individually with phenothiazine at the beginning of the grazing season and maintained thereafter upon the same phenothiazine-salt mixture but placed upon a pasture occupied the previous month by parasitized, untreated sheep, made a total gain of only 10 lb. in the same period. Fourteen of these animals died and 3 others were sacrificed because of debility. Losses in this group undoubtedly would have been much larger if they had not been treated individually with phenothiazine and placed on clean pasture. Postmortem examination of 20 representative lambs indicated this group to be heavily parasitized.

It is concluded, therefore, that under the conditions of this experiment, a 1:14 phenothiazine-salt mixture markedly protected the sheep from excessive parasitism when the animals, relatively free from internal parasites at the beginning of the trial, were maintained without directly or indirectly contacting other sheep. The same phenothiazine-salt mixture did not, however, protect sheep when they were placed upon a pasture recently contaminated by parasitized, untreated sheep.

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Ulcerative Lesions of the Bovine Rumen and Their Possible Relation to Hepatic Abscesses

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BESIDES occasionally ending in fatal illness, the condition known to meat inspectors as "liver abscess" has long necessitated the condemnation of a large number of beef livers, with a consequent economic loss of substantial proportions. Figures of the Federal Meat Inspection Service for 1942, summarized by the National Live Stock Loss Prevention Board,[1] show that 617,919 beef livers were condemned because of abscesses. This amounts to 5.25 per cent of all cattle slaughtered. While this is the percentage for the United States as a whole, there is considerable variation in different parts of the country, the Denver market showing the highest loss, where the livers of 13.3 per cent of all cattle slaughtered were condemned. The cities of the Pacific Coast had from 10.7 per cent to 11.7 per cent condemnations; Fort Worth was the lowest with 2.5 per cent.[2] [The Atlantic Coast region was not reported separately.] If the livers are worth about $3.00 each, the annual loss amounted to about 2 million dollars. These figures did not include the considerable number of animals slaughtered without federal inspection.

Whether these lesions are true abscesses (*i.e.*, purulent) or are more properly designated nodular necroses (Hutyra and Marek),[3] the general principles of pathology suggest that the infection necessary to cause such processes comes most frequently *via* the portal circulation. Actual study of the condition and special circumstances accompanying it in cattle, however, have been very limited.

REVIEW OF LITERATURE

Newsom[4] made thorough studies upon the bacterial flora of these lesions, finding *Actinomyces necrophorus* the sole or chief invader in almost every lesion. Similar bacteriologic results were demonstrated by Cooper, Elliot, and Woodman[5] in England.

Pellegri,[6] on the other hand, concluded from researches on "numerous bovine livers taken from the abattoirs at Milan and Tortone," Italy, that the prevailing organism in each abscess is *Bacterium* (*Corynebacterium*) *pyogenes*, together with secondary staphylococci and streptococci. He does not mention the use of anaërobic cultures, which may account for not finding the necrophorus organism, although it would seem that the smears, which are described, should have revealed that filamentous pathogen, if present. He further refers to the work of three different investigators, Bianchi,[7] Kunnelmann,[8] and Lisi,[9] who also found *B. pyogenes* to be the causative agent of liver abscesses. The hepatic lesions, as Pellegri describes them, appeared identical to those in this country.

Rubarth,[10] reporting from the veterinary college, Stockholm, describes 8 cases of hepatic abscesses and 3 of related epiphrenic abscesses, in which perforation of the posterior vena cava had occurred. Bacteriologic examinations showed *B. pyogenes* in 4 cases, streptococci in 2, staphylococci in 2, and mixed infection with *B. pyogenes*, streptococci, staphylococci, necrobacilli, and *Bacterium coli* in 3 cases. Two additional cases of like nature, referred to him by others, showed a pure infection with the necrophorus organism.

Yamamoto[11] reports 17 cases of multiple or single hepatic abscess in each of which the necrophorus organism was isolated exclusively except for the concomitant presence of a gram-positive bacillus in one case and of a coccus in another. He states that the condition is relatively frequent in Japan, at least in recent years.

McGregor[2] gives an interesting picture of the clinical symptoms of hepatic abscesses in a steer shipped from North Dakota and slaughtered in Tacoma, Wash. He describes typical nodular necroses in the liver and states that the diagnosis of necrobacillosis was confirmed by laboratory examination.

Ghinelli[12] reports a rare case of chronic peptic ulcer in the abomasum of a bull, analogous to the peptic ulcer of man. The literature, however, appears to contain no references to ulcerative lesions of the rumen like those to be described herein.

Frederick[13] found that a certain proportion

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(7 to 28 per cent) of hepatic abscesses resulted from perforating, foreign bodies in the reticulum by direct extension. He also states, regarding the very prevalent suspicion against certain types of feed, such as sugar-beet products, and soybean meal, that he found no evidence to incriminate any particular component of feed. He feels that if feeding plays any part, the important factor is a heavy ration of concentrates fed over a considerable period.

Moses[14] also determined the diet of 4,000 cases of hepatic abscesses in fat cattle and concluded that correlation with the use of any one kind of feed did not exist. With these beliefs, our studies to date lead us to agree, but the purpose of this paper is to report another phase of the problem.

MATERIALS AND METHODS

Largely at the suggestion of Dr. B. F. Gooch of the federal meat inspection service, Denver, Colorado, a study was undertaken to determine whether the condition of the four bovine stomachs would afford any clues possibly bearing on the liver disease. The true stomach and, with rare exceptions, the omasum proved negative in this respect, but the rumen and, to a minor degree, the reticulum showed a variety of lesions indicative of present or past injury. These organs were examined in detail in 1,807 cattle at the time of slaughter. The presence or absence of hepatic abscesses likewise was determined in each animal.

In each animal having abscesses (or nodular necroses) in the liver, microscopic sections were made from one or more abscesses and from the lesions in its rumen when such existed. The same procedure was followed in a number of rumens not accompanied by hepatic abscesses, although ordinarily such rumens were simply recorded as to the presence or absence of pathologic lesions, and no tissues were saved. Each block of tissue was stained by hematoxylin-eosin for histopathology, as well as by a modified Gram stain and by eosin-methylene-blue in order that any microörganisms present might be studied as to number and morphology.

DESCRIPTION OF LESIONS IN THE RUMEN

The lesions found in the mucosa of the rumen were placed in 8 different categories, although there was excellent ground for considering them all inter-related. Most of them were almost certainly different stages

Fig. 1—Class 1: The lesions designated "adherent contents."

Fig. 2—Class 2: Denuded and depigmented area accompanied by scar.

of the same process, as was often attested by finding more than one kind closely associated in the same rumen.

Lesions of class 1 were designated simply as "adherent contents." When such a rumen was washed out, a strong stream of water being used for this purpose in the routine packinghouse process, there remained patches of black or yellow and black material adherent to the mucous surface. These patches were round or oval and commonly about 2 cm. in diameter. They occurred in groups involving an area perhaps 10 to 30 cm. in diameter located in the smoother, slightly papillated parts of the rumen, chiefly the mid-dorsal wall. The adherent material could be scraped off with varying degrees of ease or difficulty, leaving a mucosal surface which appeared only slightly damaged, but the uncovered mucous membrane was almost always completely depigmented, with villi at a minimum.

Microscopically, the changes were often considerable. The mildest were characterized by hyperkeratosis of the epithelium and a scattering of leucocytes in the underlying connective tissue. In some cases, whole villi may have undergone coagulative necrosis with patches of their desquamated epithelium forming part of a thin layer of fibrinonecrotic exudate unsuspected at the gross examination. Heavy infiltrations of leucocytes invaded the connective tissue stroma arrested only by the edge of the muscularis. Changes in nearby epithelium were not limited to hyperkeratosis but (regenerative) hyperplastic tendencies of the deeper cells (stratum germinativum) sent bizarre "rete pegs" projecting deeply into

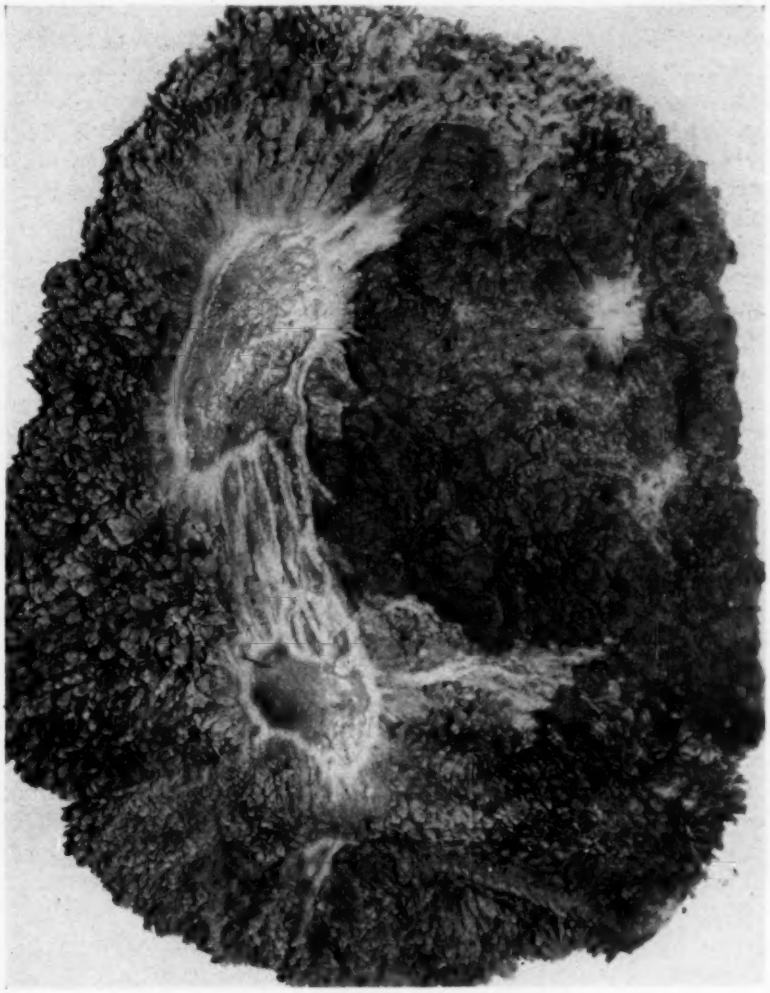

Fig. 3—Class 4: Diphtheritic exudate (two dark areas without normal papillae), adjacent to two ulcers in the process of healing.

the interpapillary connective tissue of the lamina propria. In augury of early healing, there was sometimes seen a very thin layer of regenerating epithelium pushing its way across the defect beneath the necrotic exudate.

Numerically, this class included 6.9 per cent of all lesions. They were found at least once in close association with lesions of classes 2, 3, 5, and 7.

In class 2 were placed "denuded, depigmented, and eroded" areas. They were located in either smooth or papillated regions and were of any shape and size, ranging from 2 or 3 to many square centimeters in greatest diameter. Such spots were sharply demarcated, without pigment, and often with the villi missing, quite different in appearance from the surrounding pigmented mucosa. In many instances, these areas contained or adjoined places where individual, small villi were eroded to the point of being bloody at the tips or on the sides, the result of very recent injury.

The microscopic picture, as a rule, was not unlike the more severe examples described for class 1, and indicated more extensive injury and more serious infection than the macroscopic appearance suggested. Lesions of this class were believed to follow lesions of class 1 and to be potential precursors of class 3. However, in the absence of proof that the mucosa was definitely injured by depigmentation alone, these

lesions were not counted in the tabulations or recorded in any other way unless accompanied by bloody erosions or some other macroscopically positive evidence of damage. Thus limited, they constituted 14.2 per cent of all lesions. They were found associated with each of the other classes of lesion, and with especial frequency (23 times) accompanied the scars designated as class 5.

While gross changes, and often the microscopic as well, were considered mild in each of the first two classes, lesions of class 3 needed no aid from the imagination or from the microscope to be rated as highly active, infected, inflammatory processes. They consisted of ulcerated areas, often the size of the hand, covered with a thick, slimy pseudomembrane of diphtheritic exudate. The layer of exudate was of foul appearance, several millimeters in depth, and tightly adherent. When forcibly scraped off, it left bloody villi, large or small according to location, which were devoid of epithelial protection. Microscopically, the necrotic fibrinous pseudomembrane, the inflammatory and edematous infiltration of the stroma, and the attempts at epithelial regeneration differed only in degree from conditions previously described.

Numerically, these constituted 6.9 per cent of all lesions. They occurred at least once in close association with each of the other classes of lesions except class 6 (papillomatous proliferation).

Lesions of class 4 were clean ulcers in various states of healing. They were to be found in any part of the mucous surface, even upon the pillars. In shape and size, they were of great variety, the largest reaching 10 or 12 cm. in greatest diameter. The central part of the ulcer was always bright red, smooth, and shiny. While the deficiency of epithelium gave it a raw appearance, the surface was dry, free from hemorrhage and exudate. Peripherally, the red surface gave way to a smooth, white, pearly covering of newly formed epithelium. Outside of this was a mature scar with more or less contraction and wrinkling. Healing was obviously proceeding from without inward, with a scar like those about to be described (class 5) as the ultimate termination. It should be noted that these ulcers were invariably shallow, scarcely extending through the mucosa. Hence, they are not comparable to the peptic ulcer of the human stomach.

This class accounted for 9.7 per cent of all lesions, being found at least once in close association with each of the other classes.

The fifth class of lesion was simply a scar. The scars were recognized by their slightly raised, smooth, white, shiny surface and the wrinkling resultant from contraction of the healing mucous membrane. Often, the wrinkles and folds, converging at the center, produced a stellate appearance reminiscent of the scar of glanders in the equine nasal mucosa. The usual diameter, including the wrinkled portion, was 2 to 5 cm., but some were smaller and some much larger. Some scars were linear, resembling those commonly seen in the skin as the result of a well-healed wirecut. Many were very conspicuous, but some seemed to be fading out, reaching the stage where one could not differentiate between a scar and a mere temporary wrinkling of the mucosa. It seemed practically certain that many or all scars would eventually disappear completely and leave no trace of the injury of which they constituted the healing stage.

These lesions were found in all parts of the rumen. Numerically predominant, they constituted 48.2 per cent of all lesions. Instances occurred where they were closely associated with each of the other classes.

Class 6 was designated "papillomatous proliferation." These lesions were sharply projecting nodules, single or multiple, smoothly covered with mucous membrane. Some nodules exceeded a centimeter in height, but most of them were smaller. They were located in any part of the rumen but most frequently in the smoother, dorsal areas or upon a pillar. A noticeable proportion occurred in connection with, and as a part of, the scars previously described, and a few actually had smooth, red, ul-

cerated depressions at their tips. When accompanying the usual flat scars (class 5) the papilloma-like nodules were often in clusters and bunches. Gross appearance suggested that these should all be considered as exuberant granulation tissue finally covered by epithelium, and were, like the other scars, produced in the course of the healing of ulcerations. The microscopic examination supported this conclusion. The core of the papilloma-like projection consisted of young, embryonal connective tissue with the leucocytes of active inflammation still present in varying numbers. Neighboring fibrous strands of the lamina propria were often remarkably dense and hyaline, as if they had shared the chronic inflammation of healing and scarring.

The papillomatous proliferations made up 9.0 per cent of all lesions. They occurred in close conjunction with denuded, depigmented, and eroded areas (class 2), clean ulcers (class 4), scars (class 5), and clumped villi (class 7).

The seventh abnormality was briefly called "clumped villi." A group of several or a dozen of the longer villi were sticky and coherent. Thorough washing would perhaps remove some still adherent particles of ingesta but would not separate the individual villi. Microscopically, inflammatory infiltration was demonstrable, together with necrosis of the epithelium. It is believed that such injury probably is, like "adherent contents," a precursor to the series of ulcerative changes represented by classes 2, 3, 4, and 5, previously described. Whether or not this assumption is correct, the damage seems quite sufficient to permit the entrance of infectious organisms, whose subsequent course presents the possibilities mentioned before. Only 3.0 per cent of all lesions belong to this class, so that its numerical importance is not great. Instances occurred in which clumped villi were closely associated with each of the other classes of lesion.

Class 8, rather heterogeneous perhaps,

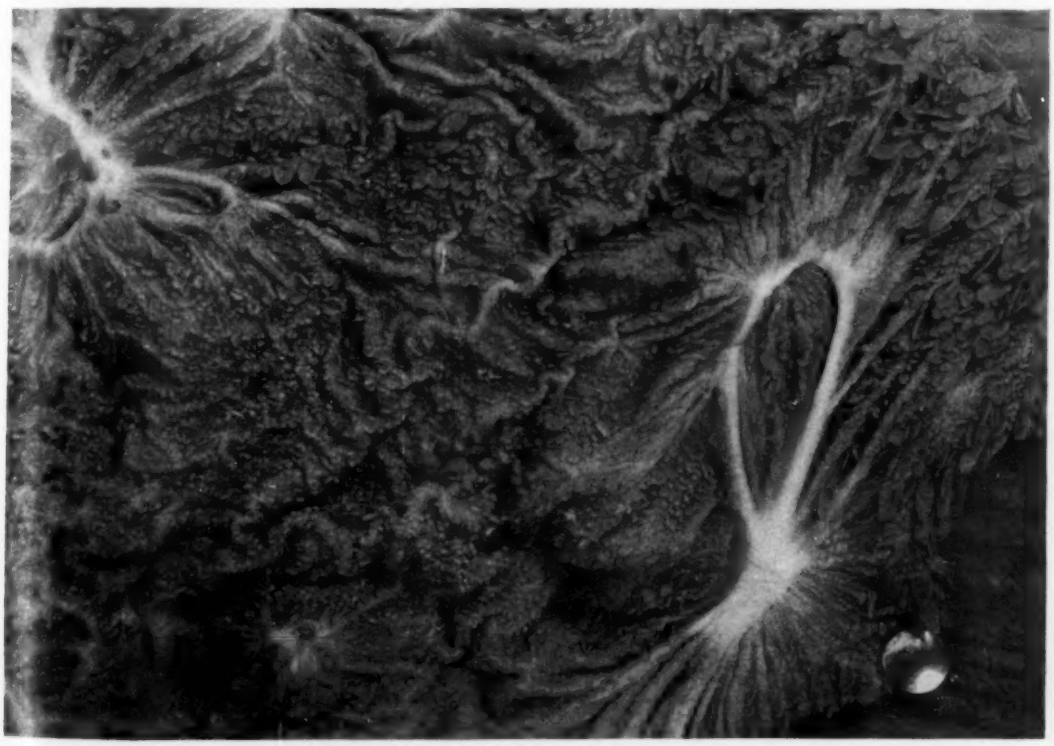

Fig. 4—Old scars within the rumen.

was termed "submucous nodules." Occurring in the smooth parts, frequently upon pillars, they were prominent, sharply circumscribed, firm or hard bodies beneath the mucosa. They were spherical or elongated and from less than 1 to more than 2 cm. in greatest diameter. A few of them proved, upon sectioning, to contain tiny abscesses. Many were nodules of dense fibrous tissue. The theory that the latter might have marked the assault of some erratic parasite is attractive but unsupported. Among all lesions, 2.2 per cent fell in this group. Instances of their occurrences with lesions of classes 2, 3, 4, 5, and 7 were noted.

RELATION TO HEPATIC ABSCESSES

The anatomical and physiological plausibility of hematogenous metastasis from the portal area to the liver is obvious. The hepatic abscesses which follow complicated appendicitis and amebic dysentery attest the facility with which such a metastasis can occur in man. An answer to the question whether certain cases of bovine, hepatic abscess might owe their origin to metastasis from the previously described lesions in the rumen can and should be sought from several different points of inquiry. A tabular summary of the frequency with which the two conditions occurred together in the same individual among 1,807 beef animals is presented here.

From the tabulation, it will be seen that 26 per cent of all cattle examined showed ruminal lesions and that 18 per cent showed liver abscesses. Of the cattle exhibiting ruminal lesions, 42 per cent had abscesses in the liver while only 9 per cent of those without such lesions showed hepatic abscesses. Conversely, of the animals showing liver abscesses, 62 per cent had ruminal lesions while only 18 per cent of those without hepatic abscesses carried lesions in the rumen.

FOREIGN BODIES

The term "damaging, foreign body" is used to designate a metallic foreign body, usually a wire or nail, which has passed through the wall of the reticulum into contiguous structures. This diagnosis was given if such a body were actually found or if its existence were revealed by a fistulous tract, a local abscess, or a local mass of granulation tissue. On the other hand, it was not unusual to find a nail or wire extending through one of the folds of the mucosa of the recticulum as well as small scars on the folds such as would have resulted from some superficial injury. In these cases there was no penetration of the

Cattle Showing Ruminal Lesions

Total cattle	No. with ruminal lesions	Per cent with lesions
1,807	474	26

Cattle Showing Hepatic Abscesses

Total cattle	No. with abscesses	Per cent with abscesses
1,807	322	18

Cattle With Ruminal Lesions Showing Hepatic Abscesses

No. with ruminal lesions	No. with abscesses	Per cent with abscesses
474	201	42

Cattle Without Ruminal Lesions Showing Hepatic Abscesses

No. without ruminal lesions	No. with abscesses	Per cent with abscesses
1,333	121	9

Cattle With Hepatic Abscesses Showing Ruminal Lesions

No. with abscesses	No. with ruminal lesions	Per cent with lesions
322	201	62

Cattle Without Hepatic Abscesses Showing Ruminal Lesions

No. without abscesses	No. with lesions	Per cent with lesions
1,485	273	18

wall and no injury or reaction deeper than the fold of mucosa. These were ignored in the tabulations although they might perhaps have been included with the ruminal lesions since their relation to hepatic abscesses must be very much the same.

Of the 1,807 cattle, 35 had damaging foreign bodies. Twenty-one of these were in animals which had hepatic abscesses; the remaining 14 were in animals with healthy livers. To put it another way, perhaps with more significance, it can be said that, of the 322 cattle with abscessed livers, 21, or 6.5 per cent, had damaging foreign bodies, although 8 of them at the same time also had other lesions in the rumen, and it would not be known to which source, if either, hepatic metastasis should be attributed. Of the 1,485 cattle without hepatic abscesses, 14, or slightly less than 1 per cent, had damaging foreign bodies, none of them accompanied by ruminal lesions.

That the incidence of foreign bodies was lower in this study than has been the experience of some other investigators is possibly attributable to the kinds of cattle involved. Foreign body disease is usually an affliction of farm or dairy cows, which have had a longer life and closer contact with barnyard surroundings, so that their opportunity for ingesting metallic objects is much greater than that of the typical range-raised beef animal.

TYPES OF CATTLE

The cattle covered in this study were predominantly fat beeves, mostly Herefords. They included whatever passed over the killing floor at a particular day and hour, but a special effort was made to be on hand when the best consignments from feedlots were being slaughtered. Naturally, the predominance of this kind varied with the season as well as from day to day. Circumstances did not permit ascertaining the type and quality of each animal whose organs were examined; nevertheless, this information was obtained with respect to 208 of those from which tissues were saved for microscopic examination. Of this number, 180, or 86.5 per cent, were fat steers or heifers whose quality indicated a rather complete finishing in the feedlot. The remainder were classed as "grass-fat" steers, heifers, or cows (20), and as old cows or dairy cows (8). Similar percentages would doubtless hold true for the whole 1,807 head.

SEASONAL VARIATION

Figures furnished by the federal meat inspection service at Denver indicate that liver abscesses are most frequent in cattle marketed from September to January, the peak being in November. Our observations were begun in August, 1942, and continued to September, 1943, thus covering a little more than a year. While there was some variation from month to month in the frequency of the ruminal lesions, sufficient data has not yet been accumulated to indicate whether any significance should be attached to these differences.

BACTERIOLOGIC FINDINGS

The bacteria found in the stained sections of hepatic abscesses and ruminal lesions morphologically were chiefly gram-negative filaments (*A. necrophorus?*), cocci, and diphtheroids. The types found in a given animal were usually similar in both liver and rumen.

By way of bacteriologic digression from the usual method of examination, 5 lesions of class 3 (foul, diphtheritic areas) were submitted to cultural examination. Material from beneath the pseudomembrane was inoculated upon aërobic blood-agar plates and into anaërobic brain broth. Only 1 of the plates showed significant growth, a hemolytic diphtheroid (*Corynebacterium pyogenes?*). All the anaërobic cultures showed gram-negative filaments typical for *A. necrophorus*, and all 5 also contained numerous streptococci. Four of the 5 contained sporulating bacilli. Diphtheroids were found in 1 of these four anaërobic cultures.

SUMMARY AND CONCLUSIONS

A variety of pathologic lesions in the rumens of beef cattle is described. It is suggested that all of these represent a series

of steps in the development and healing of superficial ulcers.

Various data are presented and discussed by comparing the incidence of these ulcerative lesions with the incidence of hepatic abscesses in a series of 1,807 animals.

The evidence presented tends strongly to justify the belief that there is a definite relationship between ulcers of the rumen and abscesses of the liver in beef cattle.

ACKNOWLEDGMENTS

In addition to Dr. B. F. Gooch, U. S. inspector in charge of meat inspection Denver, Colo., whose valuable suggestions and assistance have already been acknowledged, the writer wishes to thank particularly the various members of the meat-inspection staff, who did not hesitate to augment their routine duties in order to make this study possible. Among them are Drs. I. L. Barstow, C. M. Chase, W. A. Decker, W. D. Fountain, H. H. Groat, G. C. Harrington, P. S. Moe, W. J. Mulroony, R. H. Pease, H. L. Shorten, and W. J. Snodderly.

While all meat packers of Denver are coöperating in the general investigation, the work here reported was done in the plant of Swift and Company, and the writer is grateful for the helpful interest and the willingness to be inconvenienced shown by all members of the organization.

It is also desired to mention the assistance of the Animal Husbandry Department, Colorado State College.

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Swine Erysipelas Organisms Recovered from a Brown Rat (Rattus norvegicus)

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MEDICAL LITERATURE contains numerous references to the prevalence of swine erysipelas organisms, *Erysipelothrix rhusiopathiae*, and their recovery from various species of lower animals, including swine, sheep, fowl, and fish. In man the infection is known as "erysipeloid" in order to distinguish it from human erysipelas, which is caused by a hemolytic streptococcus. A similar species, *Erysipelothrix murisepticae*, was discovered by Koch in mice (1879) as the cause of mouse septicemia. Recently, Van Es[1] expressed his opinion that the organism we found in a rat was undoubtedly the swine erysepelas bacillus, notwithstanding there is no record in the literature of such findings previously. He referred also to Poels' suspicion of wild rodents as reservoirs of the bacillus.

While Bergey[2] describes three separate species, *Ery. rhusiopathiae, Ery. murisepticae*, and *Erysipelothrix erysipeloides*, as belonging to the genus Erysipelothrix, other authorities, including Merchant,[3] are unable to show any appreciable cultural or serological differences between them. Hagan[4] states the microscopic and cultural features of these organisms are identical but their pathogenicity differs.

Fig. 1—Erysipelothrix rhusiopathiae—rat serum agar culture. x1300.

HISTORY

During the summer of 1943, a campaign of rat extermination was inaugurated by the Denver Health Department and the Fish

From the Branch Pathological Laboratory, U. S. Bureau of Animal Industry, Denver.

and Wildlife Service of the Department of the Interior. During the same period, agents of the United States Public Health Service were also active in trapping rats in Denver and vicinity for their plague investigations. The campaign resulted in the destruction of many thousands of rats by trapping, gassing, and poisoning.

About 100 of these rats were selected at random and submitted to the Denver Branch Pathological Laboratory, United States Bureau of Animal Industry, for bacteriological and other investigational studies.

Included in our findings were trichinae, liver tapeworm cysts, and a variety of microörganisms. Among the latter were streptococci and staphylococci, paratyphoid strains, *Escherichia coli*, unidentified types, and two species of organisms of special importance.

Since *Pasteurella pestis*, the organism which causes bubonic plague, has been recovered from fleas on rodents in four rural areas of Colorado, the possibility that this bacterium might also be found in rats in Denver was considered. From one rat, cultures having the biological characteristics of the Pasteurella group were submitted to the San Francisco Plague Laboratory of the United States Public Health Service for check identification. Under date of Oct. 21, 1943, their reply indicated the culture was classified as probable *Pasteurella muricida* or *Pasteurella cuniculicida* and not *P. pestis*.

The second important culture recovered from a diseased rat proved to be *Ery. rhusiopathiae*.

NECROPSY FINDINGS

The species of rat examined was identified by E. R. Kalmbach, Fish and Wildlife Service, as *Rattus norvegicus*. Its distribution includes Denver and other cities in Colorado.

This adult, female, brown rat was found on a dump near the stockyards. Because of its diseased condition the animal was easily captured. On inspection, numerous fleas were observed. Mouse inoculations with the triturated fleas proved negative for swine erysipelas.

The right hind leg and thigh of the rat were swollen, deformed and partially gangrenous. The three remaining feet were distorted and club-shaped. About 2 inches of the tail was sloughing. The right inguinal lymph node was edematous, enlarged, and hemorrhagic. The spleen was darkened, thickened, and enlarged.

ANIMAL INOCULATIONS

Portions of the spleen, inguinal lymph node, and diseased leg muscle were macerated in sterile saline solution, and 0.5 cc. were injected subcutaneously into a white mouse, which died 4 days later. Stained smears from the blood and spleen of the dead mouse revealed minute, gram-positive rods resembling erysipelas organisms. Subsequently, this bacterium proved to be *Ery. rhusiopathiae*. Additional mice and pigeon inoculations proved the rat organism pathogenic for these animals. In each instance, *Ery. rhusiopathiae* was recovered from the dead test animal. Figure 1 illustrates the organism from the diseased brown rat.

CULTURAL CHARACTERISTICS

Cultures seeded directly from the heart blood and spleen of the original dead rat proved to be *Ery. rhusiopathiae*. The growth on various culture mediums corresponded to the characteristics described in standard works on bacteriology, including the description given by Kelser and Schoening.[5] The gram-positive, slightly curved, nonmotile rod grew more abundantly on serum than on plain agar. In gelatin stabs, the test-tube brush appearance was noticeable. Slight turbidity, without pellicle formation, developed in broth culture.

The identity of the organism was further confirmed by Giltner[6] in our Washington laboratory. He identified the culture as *Ery. rhusiopathiae* from pigeon 4892. This bird was injected with the culture originally isolated from the infected rat.

This paper presents positive evidence that at least one species of rat does carry *Ery. rhusipathiae*. Perhaps other species may also harbor the organism. Since rats are widely distributed, the possibility of the

transmission of swine erysipelas by these rodents must be given consideration.

SUMMARY

1) Swine erysipelas organisms, *Erysipelothrix rhusiopathiae*, were recovered from a diseased female, brown rat, *Rattus norvegicus*. The animal was caught on a dump near the stockyards in Denver, Colorado.

2) The discovery of swine erysipelas organisms in a rat indicates such rodents might transmit the disease from infected hog lots to noninfected premises.

3) These findings add another reason for rat control. This undesirable pest is already convicted of many crimes against public health and economy.

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A Report on Pasteurella Pseudotuberculosis Infection in Turkeys

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SEVERAL OUTBREAKS of a disease marked by anorexia, diarrhea, droopiness, lameness in a few, and sudden death of some affected birds occurred in a few turkey flocks in Oregon during the fall of 1940. Postmortem findings were not constant, although miliary, light-colored areas of necrosis and infiltration in the liver and spleen and catarrhal enteritis were the lesions most regularly noted.

From many of the birds thus affected, an organism similar to *Pasteurella pseudotuberculosis* (Bergey,[1] Gay,[2] Topley, and Wilson[3]) was isolated. Bacteriological cultures from turkeys of 6 flocks affected in the fall of 1941 yielded similar organisms. During the summer of 1942, the organism was isolated from 1, 11-week-old and 1, 4-week-old poult. The latter was concurrently infected with *Salmonella derby*.

Beaudette[4] reported the isolation of *P. pseudotuberculosis* from a blackbird. Moss and Battle[5] have reported a fatal infection with this organism in a human being in the United States. It is reported[2,7,8] to cause losses chiefly among rodents, although sporadic cases have occurred in the canary, cat, chicken, cow, horse, monkey, pig, pigeon and turkey. Beaudette's[4] comprehensive review of the literature indicated that *P. pseudotuberculosis* had never before been positively identified in connection with any disease outbreaks in birds in the United States. The isolation of organisms which appear to be identical with *P. pseudotuberculosis* from several flocks of turkeys is believed, therefore, to be of sufficient interest to warrant a report at this time. This organism may be the etiological agent in outbreaks of an hitherto undiagnosed disease in turkeys.

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At present, on leave in the Armed Forces (Rosenwald).

The authors are indebted to Dr. P. R. Edwards of the Kentucky Agricultural Experiment Station for the identification of this species.

EPIZOÖTIOLOGY

In the twenty-month period covered by this report, *P. pseudotuberculosis* infection was isolated from turkeys in 10 flocks. Of 23 birds examined from these 10 flocks, the organism was isolated from 15 birds. Incomplete data indicate that the death of at least 122 turkeys approaching marketable age and condition of a total of 13,250 birds in the flocks was due to this infection. *P. pseudotuberculosis* infection also contributed to the loss of 500 birds out of a total of 2,700 younger poults.

The disease occurred primarily in the fall. The affected birds in all but one flock were young turkeys 3 to 8 months of age. The sick birds were droopy and exhibited anorexia and diarrhea; a few became lame. A small number died suddenly in good flesh or with only slight loss of condition. Although the morbidity may be high, the mortality has been low in the cases studied. In only one instance was an outbreak observed in turkeys on the same farm in two successive years.

Epizoötiological data for the flocks from which *P. pseudotuberculosis* has been isolated are noted in table 1.

PATHOLOGICAL FINDINGS

The lesions most generally noted were soft, necrotic, or infiltrated miliary areas in the liver and sometimes in the spleen. One bird had a greatly enlarged liver with massive areas of cellular infiltration. Discoloration of the kidneys, icterus, and catarrhal or hemorrhagic enteritis were also noted. Areas of grayish infiltration were

found in the pancreas and in the intestinal submucosa in some cases.

It was not possible to demonstrate organisms in all birds examined. When isolated, the organism was generally recovered from the liver, and less frequently from the spleen and heart's blood.

STUDIES OF THE ORGANISM

The organisms isolated from sick or dead turkeys were gram-negative, round-ended rods which evidenced some bipolar staining. When grown at room temperature in dextrose broth they were feebly motile. When grown on other mediums and under other conditions no motility was noted. In broth, the organisms produced flocculi which settled out after forty-eight to ninety-six hours. Gelatin-nutrient-agar, enriched with peptone and Difco tryptose phosphate broth, proved good mediums for isolation and propagation of the bacteria.

These organisms grew more readily at room than at incubator (37 C.) temperature. On primary isolation, some strains grew only at room temperature. In many instances, there was a 24- to 48-hour interval before growth was noted on solid medium. On gelatin-nutrient-agar slants the organism formed colonies that were grayish white, slightly raised and moist, but after several days they became confluent.

The strains isolated readily fermented dextrose, mannitol, arabinose, xylose, mannose, and levulose without gas. Maltose, galactose, and, by some of the strains, salicin were fermented more slowly, also without gas. Lactose, dextrin, dulcitol, sucrose, and raffinose were not changed.

None of the strains tested produced indol. Most of the strains produced hydrogen sulfide, although they were weak in this respect. The strain isolated from accession 421-41 produced hydrogen sulfide in original test but after passage through a guinea pig and reisolation, hydrogen sulfide was not produced.

TABLE I—Epizoötiological Data on Outbreaks of a Disease Caused by Pasteurella Pseudotuberculosis

DATE EXAMINED	NO. IN FLOCK	AGE (WEEKS)	NUMBER EXAMINED	POSITIVE CULTURES	NUMBER DEAD*	REMARKS
11/12/40	1500	20	2	2	17	Ac. 293-41 & Ac. 299-41—All losses but one were in males.
11/15/40	500	27	1	1	13	Ac. 298-41—Losses only in males.
2/28/41	500	44	2	1	6	Ac. 421-41—Hen from which organism was isolated had enlarged liver due to cellular infiltration.
10/21/41	4000	14-18	6	2	35	Ac. 268-42, 279-42
11/4/41	950	18	3	2	14	Ac. 302-42 (same owner as Ac. 298-41)
11/7/41	3700	14	2	1	15	Ac. 305-42—No further report.
11/13/41	800	26	2	2	10	Ac. 317-42—Losses continued after examination.
11/24/41	1300	24	2	2	12	Ac. 328-42—No further report. All losses were in males.
6/3/42	1300	11	1	1	300	Ac. 671-42† cumulative loss since hatching.
6/3/42	1400	4	2	1	200	Ac. 671-42† cumulative loss since hatching. *Salmonella derby* infection concurrently.

*Mortality reported at time of examination.
†Poults brooded and ranged concurrently by same owner.
Ac. = accession.

The organisms survived inoculation into tetrathionate broth (Difco) and could readily be recovered from this medium by plating on MacConkey's agar. The colonies on the plates resembled those of *Salmonella pullorum*. When inoculated into cysteine-gelatin medium,[6] the 4 strains used did not cloud this medium nor form any flocculi. Some strains when inoculated into tryptose phosphate broth (Difco) grew for a few days but could not be recovered after ninety-six hours of incubation.

None of the strains of the organism

cross-agglutinated with *S. pullorum* antiserum.

PATHOGENICITY OF THE ORGANISM

A limited number of animal inoculations further confirmed the identification of the organisms as *P. pseudotuberculosis*. Tryptose phosphate broth cultures of the organism isolated from accession 298-41 were injected into 2 guinea pigs, 1 cc. each, intraperitoneally, and death ensued in less than seventy-two and ninety-six hours, respectively. Pin-head areas of grayish liver necrosis and infiltration, as pictured by Moss and Battle,[5] and excessive gastric mucus were noted on postmortem examination. Pure cultures of the organism were isolated from the liver, heart's blood, and spleen of the dead, test animals.

One guinea pig fed 5 cc. of an eight-day-old broth culture of one of the strains recovered from accession 421-41, died after a week. Another died thirteen days after receiving 2 cc. of the same broth, intraperitoneally. Typical lesions were noted in each and pure cultures of the organism were recovered.

Using a strain isolated from accession 671-42, 1 cc. of a 72-hour broth culture injected intraperitoneally killed a guinea pig in six days. Two cc. of this same broth, injected subcutaneously, was lethal for a guinea pig in ten days. Both animals exhibited purulent pneumonia and characteristic hepatitis. Pure cultures of the organism were recovered. After guinea-pig passage, this strain, grown in tryptose phosphate broth for twenty-four hours and administered to 2 turkey poults, 2 cc. to one subcutaneously, and 30 cc. by mouth to the other failed to prove lethal. Since the growth was scant in the inoculum, it may be that a sublethal dosage was used.

A suspension of liver from an infected turkey was administered by mouth, intraperitoneally, subcutaneously, and intravenously to a young healthy turkey. It apparently caused transitory illness, but the bird survived. When the subject bird died four months later with visceral gout, no organisms were recovered on bacteriological examination.

Three White Leghorn pullets about 6 months old were injected with a fifty-hour culture from accession 298-41, which killed guinea pigs. One pullet, receiving 0.5 cc. intravenously, died in thirty-eight hours with septicemic lesions. Pure cultures of the organism were recovered from the heart's blood, spleen, liver, and bone marrow. Another bird received 0.25 cc. into the wattle, subcutaneously. The injected wattle sloughed off, but the bird survived. The remaining pullet was fed 2 successive doses of the culture, totaling 50 cc. She became droopy, somewhat emaciated, but survived and recovered.

The organisms used for animal inoculation were uniformly pathogenic for guinea pigs; their pathogenicity in other laboratory animals was not checked. *P. pseudotuberculosis* is reported[5] to be uniformly pathogenic for white mice and guinea pigs, to cause local lesions but few deaths in rabbits, and to exert little effect on the markedly resistant white rat.

DISCUSSION

No studies were conducted to determine the source of the infection for the flocks involved. Since most of the cases occurred during the rainy season, it is suspected that puddles of water that might have been contaminated, and from which the birds drank freely, were the most likely source.

A comparison of the organism studied, with Beaudette's[4] strain of *P. pseudotuberculosis* reveals a great similarity. Beaudette reports slight acidity in dextrin and the organism studied consistently gave no change with this carbohydrate. The only other variation was the irregular slow fermentation of salicin. These slight variations do not seem sufficient to consider this organism other than *P. pseudotuberculosis*.

On the other hand, the *P. pseudotuberculosis* isolated from a human being by Moss and Battle[5] produced an acid reaction in sucrose in forty-eight to seventy-two hours that reverted to an alkaline reaction in about a week. Another striking difference was that their organism caused no change with either maltose or mannitol.

Whether the organisms form an exotoxin or not and whether they cause many of the unexplained cases of anorexia in range turkeys has not been determined.

In no instances have extremely high losses been reported, but they have occurred at a time when the value of the individual bird has been near its peak. In one flock, losses occurred due to decrease of body weight and mortality over a period of six weeks, but from only one bird of several examined was the organism isolated. The chief loss was that of body weight which is expensive to regain in birds that are near market age.

Control measures suggested have been to exercise greater care in maintaining good sanitation and a clean water supply, and to feed mash or pellets moistened with mineral oil for brief intervals once or twice each day. Any palliative to relieve the anorexia seems to be a desirable course to follow.

SUMMARY

The isolation of an organism believed to be *Pasteurella pseudotuberculosis* from sick or dead turkeys from 10 different flocks in Oregon is reported.

The identification of the organism is based on the biochemical, morphological, and physiological characteristics, and its pathogenicity for guinea pigs, chickens, and turkeys.

It is believed that this organism is concerned with the etiology of the disease noted in these flocks of turkeys. The possible importance of this infection in turkeys is noted. This infection in turkeys apparently has not been reported previously in this country.

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Studies of the Spleens of Hyperimmunized and Immunized Swine

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IN A PREVIOUS publication,[1] the histopathological alterations in the spleens of cholera-infected swine were presented. Similar lesions were observed by Seifried and Cain[2,3] in this country and by Rohrer[4] and others in Europe. More recently, DeKock, Robinson and Keppel[5] reported outbreaks of an infectious disease of swine in South Africa which they demonstrated to be hog cholera (swine fever). These investigators also found histopathological alterations of the spleen which were essentially the same as those produced by strains of the hog-cholera virus in America and Europe. Besides being of value as a means of differential diagnosis, these studies are useful in our understanding of the fundamental nature of this disease.

While the histopathology of the spleen of cholera-infected swine has been well established, apparently little attention has been given to the histological changes in the spleen of hyperimmunized swine. Likewise, there are inadequate descriptions of the spleen of immunized swine, especially in pigs that have been recently vaccinated with serum and virus. The primary purpose of this study, therefore, was to record the histopathological findings in the spleen of these classes of swine.

MATERIALS AND METHODS

Histopathological and bacteriological examinations were made of the spleens of the following classes of swine:

1) Spleens of hogs hyperimmunized against hog-cholera infection in the commercial production of anti-hog-cholera serum.

2) Spleens of young pigs recently immunized against hog-cholera with antiserum and virus.

3) Spleens of market-age hogs that had been vaccinated using hog-cholera antiserum and virus in the usual manner at weaning age.

Spleens from four lots of hogs numbering 137 animals comprised the hyperimmunized group. These were garbage-fed hogs and, consequently, were somewhat older than grain-fed hogs of the same weight. Experience has shown that garbage-fed swine are more suitable for hyperimmunizing purposes. The hyperimmunization was conducted according to the standard procedure adopted by the United States Bureau of Animal Industry. Hogs selected for hyperimmunization had been immunized against cholera for at least ninety days. Each animal received intravenously 5 cc. of virulent, hog-cholera blood per pound of body weight. There were four bleeding periods seven days apart beginning ten days after the hyperimmunizing injection. The spleens were obtained at the final bleeding period or thirty-one days following the injection of the virus.

A group of 12 weaned pigs, weighing about 35 lb., was obtained for a study of the spleens of recently vaccinated animals. Each pig had received 30 cc. of antiserum and 3 cc. of virus. Three pigs of this group were destroyed at weekly intervals beginning the first week following the administration of serum and virus.

Spleens were obtained from 65 market-age hogs which represented a normal class of animals for this study. These animals had been vaccinated shortly after weaning when approximately 2 months of age. They were sent to market when 7 months old and at an average weight of 225 lb. During the nursing period, this group had access to rye pasture when available. The average weight at weaning was 30 lb. From weaning until the weights reached 70 lb., the ration consisted of alfalfa pasture and a grain mixture consisting of 70 lb. of corn, 20 lb. of wheat and 10 lb. of tankage. The hogs were finished for market with corn, pasture when available, and a protein supplement as follows: 20 lb. meat and bone scraps, 20 lb. fishmeal, 40 lb. soybean oil meal, 10 lb. linseed oil meal, and 10 lb. cottonseed meal. When pasture was not available, alfalfa leaf meal replaced the linseed oil meal in the protein supplement.

The weights of the spleens of 121 animals from the first 3 lots of hyperimmunized hogs and from the first lot of 40 market-age hogs

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were recorded. Since individual body weights were not available, the splenic weight in grams per kilogram of body weight was computed on the basis of the average splenic and average body weight of each lot. Computation was made with the formula $\frac{\text{splenic wt. (Gm.)}}{\text{body wt. (kg.)}}$ which Gilmore, Venske and Faust[6] used in determining the average weight of the thyroid gland per kilogram of body weight for various age groups of normal dogs.

TABLE 1—Comparison of Average Splenic Weight to Average Body Weight for Lots of Hyperimmunized and Normal Market-Age Hogs

Class	No. Examined	A.B. wt. (kg.*)	A.S. wt. (Gm.)	Range in S.W. (Gm.)	S.A. per kg. Body wt.†
Hyperimmunized					
Lot 1	34	164	233	148-341	1.420
Lot 2	40	132	194	127-275	1.469
Lot 3	47	123	175	119-289	1.422
Market-Age Hogs (Normal) .	39	102	111	59-160	1.088

A.B. = average body; A.S. = average splenic; S.W. = splenic weight; S.A. = splenic average.
*Range in body weights was not available.
†According to the formula $\frac{\text{spleen weight (Gm.)}}{\text{body weight (kg.)}}$ using average splenic and average body weight of each lot.

Bacteriological examinations were made on meat-infusion and liver-infusion agar from each spleen of three lots of hyperimmunized hogs numbering 126 animals. Similar cultures were made with the spleens of the 12 recently vaccinated pigs and with the spleens of 40 market-age hogs. In order to obtain a larger representation of tissue in the bacteriological examination, the spleens of 11 hyperimmunized and 25 market-age hogs were examined as follows: From 1 to 2 Gm. samples of splenic tissue were ground with sterile, quartz sand in a mortar containing 5 cc. of physiological salt solution. The fluid portion was placed in large tubes or flasks with not less than 35 cc. of tetrathionate broth (Difco) and incubated at 37.5 degrees C. for twenty-four hours. After incubation, streaks were made from each broth sample on three types of differential plate mediums including Endo's agar, MacConkey's agar, and S-S agar (Difco). All bacterial cultures obtained were classified in the standard manner on the basis of reactions on appropriate carbohydrate mediums.

In this study, spleens were obtained only from hog carcasses that were found free from tuberculous infection during the post mortem examination. However, in an effort to still further eliminate the possibility of this infection, smears from the spleens of two groups of hyperimmunized hogs numbering 88 animals and also from the spleens of 40 market-age hogs were examined microscopically by means of the Ziehl-Neelsen acidfast stain.

For histological study at least two portions of each spleen were fixed in formol solution. Sections prepared from these tissues were stained routinely with Harris' hematoxylin and eosin. Special stains, such as methyl violet 6B, followed by differentiation in one per cent acetic acid solution, were used in examining for amyloid. This stain was applied to paraffin sections of the spleens of 45 hyper-

TABLE 2—Bacteriological Examinations of Spleens of Various Classes of Swine

Class	No. Examined	Cultural Mediums (Agar)	Reactions on Carbohydrate Mediums: N. G.	Salmonella Cholerae-suis Types	Coliform Types	Non-coliform Types	N. C.	Acidfast Stain: No. Examined	P	N
Hyperimmunized										
Lot 1	34	Liver & beef	24	0	0	7	3	0	..	..
Lot 2	45	"	30	0	0	13	2	41	0	41
Lot 3	47	"	38	0	0	9	0	47	0	47
Lot 4	11	Differential Plate	5	0	0	6	0	0	..	..
Recently Vaccinated:										
Lot 1	12	Liver & Beef	11	0	0	0	1	0	..	..
Market-age Hogs (Normal)										
Lot 1	40	Liver & Beef	25	0	4	15	0	40	0	40
Lot 2	25	Differential Plate	11	0	10	10	0	0	..	..

N.G. = no growth; N.C. = not classified; P = positive; N = negative.

immunized hogs. In addition, frozen sections of spleens from lots of 11 hyperimmunized and 25 market-age hogs were stained with methyl violet and examined for amyloid.

RESULTS

While there was considerable variation in the weights of the individual spleens in 3 lots of hyperimmunized hogs, the relation of the average splenic weight to body weight was approximately the same in each lot (table 1). The average weight of the spleens of this class of hogs closely corresponds with the splenic weight given for normal adult swine (200 Gm.—Sisson-Grossman.[7] The weights of the spleens of 40 market-age hogs also showed considerable variation. In this lot, the average splenic weight in relation to body weight was somewhat less than in the hyperimmunized hogs. However, neither the comparative size nor the histological examination indicated that the process of hyperimmunization caused hyperplasia of the spleen.

The results of bacteriological examinations as indicated in table 2 did not reveal any significant bacterial infection in the spleens of the different classes of swine. Only one culture, isolated from the spleen of a market-age hog, was suggestive of being a Salmonella type on the basis of reactions on carbohydrate mediums. Examination of this culture by Dr. D. W. Bruner of the University of Kentucky showed that it was not a Salmonella type according to the Kauffman-White schema for the classification of this genus. The splenic smears of 2 lots of hyperimmunized hogs numbering 88 animals and also of 1 lot of 40 market-age hogs did not show evidence of acid-fast bacteria.

At autopsy, there were 18 spleens out of three lots of 126 hyperimmunized hogs that showed diffuse dark red areas on the margin of the organ. Much smaller areas of this type were observed in nine spleens out of 12 recently vaccinated pigs. In each instance, however, the gross appearance of these areas was not typical of the splenic lesions of cholera-infected swine, which are characterized by circumscribed, slightly elevated dark areas that have pale centers suggesting necrosis.

Histologically, a considerable number of the spleens of hyperimmunized hogs in lots 1 and 3 showed degenerative changes of the walls of the blood vessels. Karolysis of the splenic cells was observed, especially in the lymphoid follicles. The degenerative changes were much more extensive in lot 2, in which complete hyalinization of the walls of the blood vessels was frequently found (fig. 2). The spleens in this lot also showed areas of focal necrosis which were sometimes united with adjacent foci to form larger masses of necrotic tissue (fig. 3). The arterial sheaths

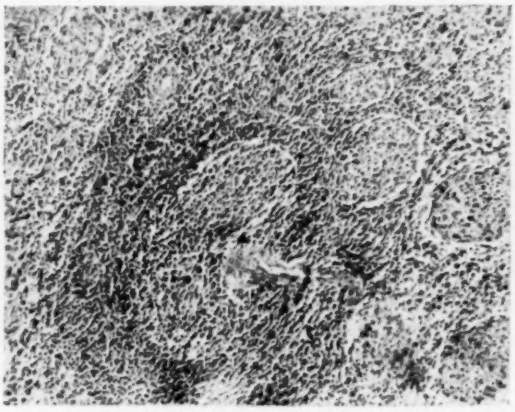

Fig. 1—Spleen of normal market-age hog showing ellipsoidal sheaths of Schweigger-Seidel around a follicle. (Approximately 100.)

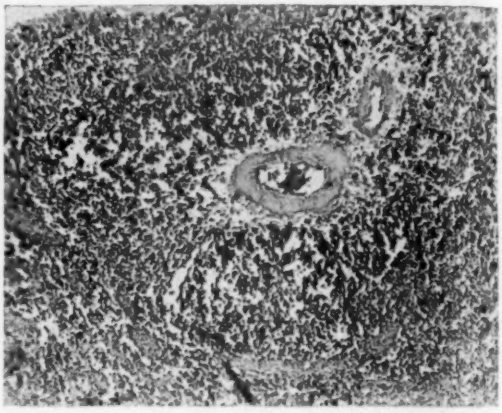

Fig. 2—Spleen of hyperimmunized hog showing hyalinization of the wall of an artery and condensation and dissolution of nuclear material. Amyloid stain negative. (Approximately 100.)

of Schweigger-Seidel,[8] which are prominent in the spleens of normal swine (fig. 1), retained their outlines except in the large areas of necrotic tissue (fig. 3 and 4).

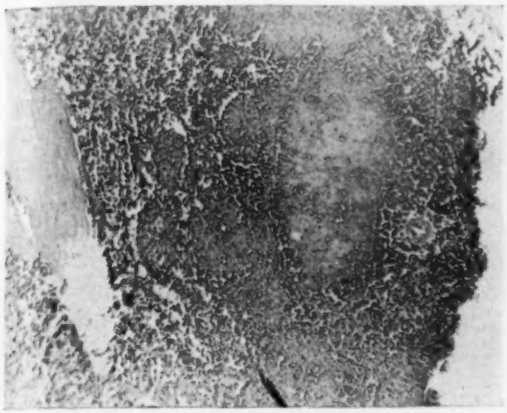

Fig. 3—Spleen of hyperimmunized hog showing extensive necrosis in area of a follicle. The Schweigger-Seidel sheaths around the follicle are practically obliterated. (Approximately 100.)

Hyalin changes in lot 2 of the hyperimmunized hogs involved not only the blood vessels but also the trabeculae, resulting in a glassy appearance of these structures (fig. 5). The methyl violet stain, however, did not indicate that this hyalin material was amyloid. The same stain when applied to frozen sections of the spleens of the 11 hyperimmunized hogs in lot 4 did not show amyloid formation. The spleens of this lot were also negative to the iodine test for amyloid when it was used on the fresh specimens.

Vascular and necrotic lesions were not demonstrated in the spleens of the 12 pigs destroyed from one to four weeks following vaccination with antiserum and virus. Likewise, the spleens of the first lot of 40 market-age hogs were free from histological lesions. The spleens of the second lot of 25 market-age hogs showed no evidence of amyloid when frozen sections of each organ were stained with methyl violet.

DISCUSSION

The type of microscopic lesions observed in the spleens of hyperimmunized hogs suggests that hog-cholera virus has a definite necrotizing effect which may occur in the complete absence of secondary bacterial infection. Since this type of hog has usually been exposed to a variety of swine diseases, the tissue changes observed were more likely the result of the damage caused by the virus of hog-cholera than by other infections.

Although large areas of necrosis were not always found, the spleens of the hyperimmunized swine frequently showed con-

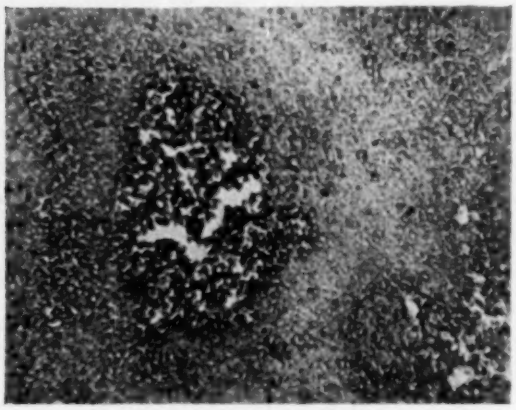

Fig. 4—Spleen of hyperimmunized hog showing zone of necrosis surrounding the remnants of a follicle. (Approximately 220.)

densation and dissolution of nuclear material of the various types of splenic cells. DeKock, Robinson and Keppel[5] showed that these changes were also present in cholera-infected swine. With reference to the condensation and fragmentation of the nuclear material these investigators stated: "This

TABLE 3—Gross and Histological Lesions of Spleens of Various Classes of Swine

CLASS	NO. EXAMINED	SUGGESTIVE LESIONS AT AUTOPSY*	HISTOLOGICAL LESIONS V.L.	HISTOLOGICAL LESIONS N.A.
Hyperimmunized				
Lot 1	34	3	13	0
Lot 2	45	7	43†	34
Lot 3	47	8	37	0
Lot 4	11	..	..	..
Recently Vaccinated				
Lot 1	12	9	0	0
Market-age Hogs (Normal)				
Lot 1	40	2	0	0
Lot 2	25	..	..	..

V.L. = vascular lesions; N.A. = necrotic areas.
*Not the well-defined infarcts of cholera.
†Lesions more extensive including hyalinization.
Note: Spleens of lots 2 and 3 of the hyperimmunized and lot 2 of the market-age hogs were negative to the amyloid stain.

change so conspicuously affecting endothelial and lymphocytic cells everywhere would seem to be the direct result of damage done by the virus and can scarcely be attributed to, or confused with, the more localized, necrotic changes that arise secondarily as

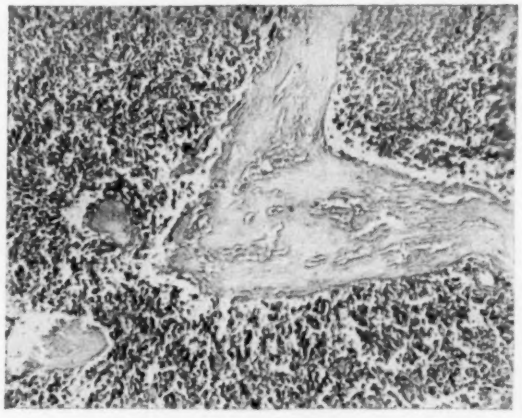

Fig. 5—Spleen of hyperimmunized hog showing a hyalinized trabecula. Note loss of cellular outlines and condensation of nuclei. Amyloid stain negative. (Approximately 100.)

a result of thrombosis and infarction."

Proliferative activity of the splenic follicles, observed in cholera, was not frequently found in the spleens of hyperimmunized hogs. Occasionally, areas of hematopoiesis were observed in hyperimmunized swine indicating efforts at regenerating red blood cells (fig. 6). This could be the result of the withdrawal of large amounts of blood following the hyperimmunizing injection.

Apparently, the hyalin material observed in the blood vessels and trabeculae of hyperimmunized hogs was not amyloid. This was of interest because there have been reports of amyloidosis in equines following certain hyperimmunizing processes. Doerkin[9] demonstrated that amyloid occurred in the spleen and also in the liver, kidneys, adrenals, and intestines of horses used in the preparation of diphtheria antitoxic serum. Recently, Weidlich[10] has reported the presence of amyloid in the liver, spleen and kidneys of horses and mules that were immunized with swine erysipelas organisms. The subjects used were mostly old animals.

Histologically, the spleens of the recently vaccinated pigs did not show evidence of damage caused by the virus of hog cholera. These findings strongly suggest that, when adequate doses of serum are used in the immunization of swine, there may be no evidence of splenic damage, even within one week after the injection of the virus.

It is of interest to note that the type of splenic lesions associated with hog cholera appeared to be similar to the multiple necrosis of the spleen that has been reported in man. This condition in human spleens was first described by Feitis,[11] who called it "Fleckmilz" on account of the speckled appearance of the organ. Recently, Schmeisser and Harris[12] have classified the cases of multiple necrosis in human spleens that

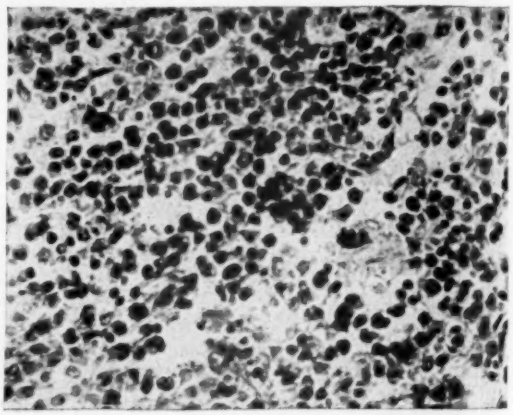

Fig. 6—Spleen of a hyperimmunized hog showing a hematopoietic center. (Approximately 440.)

have been reported in the literature into 5 groups on the basis of etiology and pathology. As in the case of swine, thrombosed blood vessels were often associated with the necrotic lesions in the human spleen. However, there were cases in man, as was observed in the hyperimmunized swine (fig. 2), in which there was extensive necrosis associated with vascular disease but there was no indication of thrombosis.

SUMMARY

The following observations are recorded in a study of the spleens of hyperimmunized and immunized swine:

1) Bacteriological examinations did not show any significant pathogenic organisms in any of the spleens of the swine used in this study.

2) Gross lesions, typical of cholera, were not present in these animals.

3) There was no evidence that the process of hyperimmunization regularly produces a hyperplasia of the spleen.

4) Histologically, the spleens of 126 hyperimmunized hogs frequently showed degeneration of the walls of the blood vessels. In 1 lot of 45 spleens there was hyalinization of the walls of the blood vessels associated with extensive necrosis of the splenic pulp and follicles. Hyalin changes also involved the trabeculae in spleens that showed the more extensive lesions.

5) There was no indication that the hyalin material in the spleens of hyperimmunized hogs was amyloid.

6) Histologically, the spleens of recently vaccinated pigs, examined from one to four weeks following vaccination with antiserum and virus, showed no evidence of hog-cholera lesions.

7) The spleens of 7-month-old hogs that were vaccinated with serum and virus at 2 months of age had a normal histological structure.

The writer is indebted to Dr. C. R. Donham and Dr. L. P. Doyle of the Department of Veterinary Science for advice and assistance; to Dr. F. N. Andrews, Department of Animal Husbandry, for photomicrographs; and to Dr. D. W. Bruner, Department of Animal Pathology, University of Kentucky, for classifying two cultures obtained from spleens of market-age hogs.

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The Rôle of Microörganisms in Reproductive Disorders of the Chicken

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IN LAYING FLOCKS, a high percentage of losses results from reproductive disorders. This condition does not represent a specific disease entity but manifests itself in numerous ways. Disorders included by the authors in this investigation were salpingitis, ruptured oviduct (rarely found), and the accumulation of one or more eggs or their component parts in variable amounts in the body cavity, oviduct, or occurring simultaneously in both.

REVIEW OF LITERATURE

The literature dealing with the possible causes of reproductive disorders was reviewed in 1938 by Moore.[1] Later work includes that by Beach,[2] who inoculated culture mediums with material obtained from a number of cases diagnosed as "ruptured yolk" with resultant peritonitis. Usually the mediums remained sterile, but several cases yielded cultures of *Escherichia coli*. He was undecided as to the etiological significance of this organism. In a study of "meat spots" in eggs, Burmester and Card[3] isolated bacteria from 4 out of 12 eggs which appeared to be of the genus Staphylococcus.

Edwards and Hull[4] isolated two cultures of hemolytic streptococci from two birds showing exudative peritonitis and salpingitis accompanied by the presence of concretions in the oviduct. The bacteria produced death when injected into other birds. Six other references to streptococcic infections in chickens were recorded by these authors.

Twisselmann[5] isolated cultures giving reactions typical of *Escherichia coli communis* from the heart blood, liver, spleen, lungs, and peritoneal contents of 17 birds. All of these birds had a rapidly fatal disease resembling fowl typhoid and fowl cholera, but in no case were the causal organisms of these diseases found to be present. The cultures of *E. coli*, when injected into 4-month-old pullets, did not cause death. However, he felt that his findings indicated that the organisms might play an important rôle in certain types of reproductive troubles in laying hens.

Hoffman and Stover[6] have used the term "ruptured yolk" to describe a condition in laying hens in which yolk material is found in the abdominal cavity. They state, "In many of these cases, the ova in well-developed ovaries have begun to be absorbed. These ova become soft and flabby, and in some instances, the yolk material leaks through the yolk membrane. In other instances, the yolk membrane ruptures permitting yolk material to enter the peritoneal cavity. It has been demonstrated that the presence of uninfected yolk material in the peritoneal cavity of healthy birds is not harmful. It would appear, therefore, that its presence in infected birds is purely incidental and should not be considered a major factor in the death of the birds."

For several years, the West Virginia Experiment Station flock has experienced heavy losses from lymphomatosis and reproductive disorders. Preliminary studies indicated a possible relationship between the two.

In 1941, a project was started, in coöperation with the Regional Poultry Research Laboratory at East Lansing, Michigan, to study this relationship more thoroughly. The purpose of this investigation was to study the bacteriology of reproductive disorders, particularly as associated with the leucosis complex.

The object of this paper is to report our findings on the rôle of bacteria as contributing factors in the etiology of reproductive disorders. During the course of the investigation, particular attention was given to those cases in which lymphomatosis and reproductive disorders were coexistent.

Birds for autopsy were obtained from an experimental flock which consisted of more than 1,000 birds. No birds were culled until they had completed their first year's production, at which time all poorer families were slaughtered. Families that had demonstrated their ability to live and produce well for one or more years were retained and the breeding flock was selected from these families. Family progeny rec-

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ords (*i.e.*, egg production, egg weight, body weight, health, and viability) were the basis for the selection of birds entering the breeding houses.

Sanitary precautions were taken to avoid losses from parasitism. All birds were found free from pullorum disease, and fowl typhoid and fowl cholera were practically nonexistent. All young stock was vaccinated annually with fowl-pox virus before going into the laying pens. Birds were confined in the laying house during the first year's production.

TABLE 1—A Comparison of the Incidence of Bacteria Isolated from Birds with and Without Reproductive Disorders

	No.	Percentage	No. Yielding Bacteria	Percentage Yielding Bacteria
Reproductive Disorders	274	26.1	122	44.5
Without Reproductive Disorders	777	73.9	379	48.8
Total	1051	100	501	....

All birds removed from the pens, either because of death or a moribund condition, were examined *post mortem* and cultured as soon as possible after death. Birds dying on Sundays and holidays were brought to the laboratory on the following day.

Before the autopsy, the birds were dipped in a 1:1000 bichloride of mercury solution, to which a wetting agent had been added and other aseptic precautions were taken to prevent contamination of the tissues to be cultured. The skin was slit with hot scissors parallel to the breast bone and pushed down along the side of the body. The exposed surface was swabbed with a fresh 1:1000 bichloride of mercury solution before cutting into the body cavity with hot scissors. All organs cultured were seared over the surface before introducing the sterile inoculating needle. Organs routinely cultured were the heart blood, liver, and spleen. Other organs were cultured when deemed advisable.

Veal-infusion agar was the medium used for making the isolations. Plates were incubated for forty-eight hours at 37 C., after which representative colonies were selected for the isolation of pure cultures. The tests used in classifying the organisms isolated were classified on the basis of morphologic, cultural, and physiologic characteristics. The latter included study of the reaction of the organisms to Gram's stain, indol production, nitrate reduction, liquefaction of gelatin, acid production in litmus milk, and fermentation of sugars. When deemed advisable, bacterial action on erythrocytes was determined by the use of blood-agar plates. In all instances, tests were conducted according to the methods given in "The Manual of Pure Culture Studies of Bacteria."[7] The identification of the species was made according to "Bergey's Manual of Determinative Bacteriology."[8]

In an effort to determine the pathogenicity of the bacteria, many cultures were maintained for inoculation experiments. For inoculations, 24-hour agar slant cultures were suspended in physiologic saline solution and introduced intravenously or intraperitoneally. The organisms used and dosage introduced are reported in table 6.

TABLE 2—Frequency of Occurrence of Organisms Isolated

Types	274 Birds with R.D. No. of I	274 Birds with R.D. P	277 Birds without R.D. No. of I	277 Birds without R.D. P
Sta. aureus and *albus*.	69	44.0	310	53.0
E. coli	60	38.2	187	32.0
B. subtilis	16	10.2	76	13.0
Str. viridans	1	.6	1	0.2
Hemolytic streptococcus	1	.6	0	0
Salmonella species	4	2.6	0	0
P. avicida	2	1.3	0	0
Others	4	2.6	11	1.2
Total	157		585	

R.D. = Reproductive Disorders; I = Isolations; P = Percentage.

Birds for experimental inoculations consisted of apparently healthy leghorn cockerels weighing between 2 and 4 lb., obtained from our growing stock. The inoculated birds were isolated from the main flock and observed. Individuals in a moribund condition or dead were taken to the laboratory and an autopsy was performed immediately. Cockerels which showed slight weakness were observed frequently for two weeks or longer, whereupon

an autopsy was performed and the birds cultured.

EXPERIMENTAL FINDINGS

During the two and one-half years that this investigation was in progress, autopsies were performed on 1,051 birds, dead or in a moribund condition, and the liver, heart blood, and spleen cultured routinely. Organisms were isolated from 501 of these birds. In table 1, a comparison is made of the incidence of bacteria in birds with and without reproductive disorders.

There were 274 birds showing evidence of reproductive disturbances, as compared to 777 which did not. The percentage of cultures isolated from birds without reproductive disorders (48.8 per cent) was slightly greater than from birds with this disease condition (44.5 per cent).

The frequency of occurrence of different types of organisms in birds with and without reproductive disorders is shown in table 2. Staphylococci and *E. coli* were the predominating types of organisms in both groups. These two constituted 83 per cent of the isolations from birds with reproductive disturbances and 85 per cent from the birds without the disorder. *Bacillus subtilis* was isolated in 10 and 13 per cent of the cases with and without reproductive disorders, respectively, but it is considered saprophytic. There were 157 isolations made from the 274 birds with reproductive disorders and 585 isolations from 777 birds without the disease. In many instances, a specific organism was isolated from more than one organ of a bird, but in this table, it was recorded as a single isolation.

The incidence of Staphylococci and *E. coli* in live and dead birds is shown in table 3. In computing these data, organisms were recorded according to the number of times they were isolated from various organs, including those cultured routinely as

TABLE 3 — The Incidence of Staphylococcus aureus, Staphylococcus albus, and E. coli in Birds Dying on the Farm, as Compared with Freshly Killed Birds, Both Groups Having Reproductive Disorders

No. of Birds	Bacteria and their sources	No. of Cultures	Percentage
27	Staphylococcus sp.*	15	55.6
247	Staphylococcus†	128	48.6
27	*E. coli**	2	7.4
247	*E. coli*†	111	44.9

* = Isolated from live birds; † = Isolated from dead birds.

TABLE 4—The Type, Number, and Origin of Cultures Obtained from Various Organs of Birds Showing Reproductive Disorders

	Site of Reproductive Disorder															
	Oviduct							Peritoneal Cavity						Ovary		Egg
	Bacteria Found															
Organ Cultured	Staphylococcus	E. coli	Str. viridans	B. subtilis	P. avicida	Salmonella sp.	Unidentified	Staphylococcus	E. coli	Streptococcus hemolytic	B. subtilis	P. avicida	Salmonella sp.	Unidentified	Staphylococcus	B. subtilis	E. coli
Liver	14	9	0	4	1	1	2	27	21	0	2	1	1	1	0	0	2
Heart	17	8	0	1	0	1	0	26	18	0	2	1	2	0	1	1	0
Spleen	15	10	0	2	0	3	0	29	25	0	2	1	1	0	0	0	0
Oviduct*	6	2	1	2	0	1	0	2	4	1	1	0	1	0	0	0	0
Peritoneal* Cavity	0	2	0	1	0	0	0	1	3	0	1	0	2	0	0	0	0
Eggs*	0	2	0	1	0	0	0	2	6	0	1	0	0	0	0	0	0
Ovary*	1	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0
Kidney*	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Total	53	33	1	11	1	7	3	89	78	1	9	3	8	1	1	1	2

*Organ not routinely cultured.

well as those cultured when special pathologic changes were present. Staphylococci were recovered from 27 birds brought to the laboratory alive, and from 247 birds dead upon arrival at the laboratory. Of the live birds, 7.4 per cent showed *E. coli* and 44.9 per cent of the dead birds yielded cultures of this organism.

The type, number, and origin of cultures obtained from birds with reproductive disorders is shown in table 4. Since the liver, heart blood, and spleen were the only tissues cultured routinely, the data in this

TABLE 5—The Type, Number, and Origin of Organisms Obtained from Routine Culture of All Birds

ORGANISM ISOLATED	ORGAN CULTURED				
	L	H.B.	S	T	P
Staphylococci	170	181	179	530	61.3
E. coli	83	85	63	231	26.7
Other organisms..	40	30	34	104	12.0
Total	293	296	276	865	100

L = Liver; H.B. = Heart Blood; S = Spleen; T = Total; P = Percentage.

table do not reflect an accurate picture of the number of times organisms might have been recovered had the other organs been routinely cultured. Some information is revealed relative to the types that may be present. As in table 3, the data were computed on the basis of the types of organisms isolated as well as the number of times they appeared in various organs. A great majority of the cultures were isolated from the organs cultured routinely; again, *Staphylococcus aureus* and *Staphylococcus albus* and *E. coli* were the predominating types present. Four birds yielded the 15 cultures of Salmonella organisms which proved not to be *Salmonella pullorum*. Two birds yielded the 4 cultures of *Pasteurella avicida*, none of which were isolated from the reproductive organs. A culture of *Streptococcus viridans* was isolated from a bird with salpingitis, and a hemolytic streptococcus was isolated from the oviduct of another bird in which the site of the reproductive disorder was in the peritoneal cavity. Four unidentified organisms were isolated.

The type, number, and origin of bacteria isolated from the liver, heart blood, and spleen is shown in table 5. The data were computed on the basis of all birds examined *post mortem* and the organisms were counted each time one or more of the routinely cultured organs yielded a specific organism. Five hundred and thirty of the culture isolated were of the genus Staphylococcus, 231 were *E. coli*, and 104 were other organisms which included *P. avicida*, Salmonella spp., Streptococci (hemolytic and nonhemolytic), and spore-forming aërobes of the *B. Subtilis* group.

Lymphomatosis and reproductive disorders occurred concurrently in 88 birds. Cultures were obtained from 47.7 per cent of the cases with both diseases, while 52.3 per cent failed to yield organisms. Staphylococci and *E. coli* were the predominating types. They were found to exist in about the same ratio as reported for the entire population.

Seventy-one birds were experimentally inoculated to study the pathogenicity of pure cultures of bacteria obtained from autopsies. Twenty-four hour cultures suspended in physiologic saline solution were introduced intravenously or intraperitoneally. The dosage varied from 0.1 to 1.0 cc. as shown in the table. Experimental birds consisted of apparently healthy leghorn cockerels weighing between 2 and 4 lbs., obtained from our experimental stock. The inoculated birds were isolated and observed for at least two weeks. Fifteen birds died; 24 sickened, of these 14 were paralyzed; and 32 showed no ill effects. Paralysis was observed in 13 or 34.2 per cent of the birds inoculated with *E. coli*, and one bird receiving Salmonella spp. was so affected. Injections with staphylococci failed to produce paralysis.

The symptoms of paralyzed birds as well as the histopathologic findings were not indicative of neurolymphomatosis, but were probably due to a toxemia. This point is being investigated further.

The sciatic nerve of one cockerel showed foci of infiltration upon histopathologic examination, but this was not thought to be associated with the partial paralysis resulting from the inoculation.

To determine the normal bacterial flora of apparently healthy birds and as a check

upon our technique, 36 hens that had just completed their first year's production were slaughtered and cultured. Twenty-two of these birds were cultured by employing extraordinary precautions to provide aseptic conditions. This consisted of killing the birds by dislocating the cervical vertebrae. Then they were dipped in hot water (60 C.) for approximately one minute, after which the feathers were removed. The skin was then dried with a clean towel and singed. A 75 per cent solution of alcohol was applied to the abdominal region prior to exposing the visceral organs, in preparation for culturing, in a dust-free room. These birds were cultured within one hour after slaughter. Fourteen birds were cultured under the routine conditions previously described, 7 of which were killed by pithing to prevent rupturing blood vessels, and then they were placed in the laboratory at room temperature for four and one-half hours before culturing. This was done to check upon the possibility of migration of *E. coli* and Staphylococci in dead birds.

The time elapsing between death and culturing (4½ hours) was not strictly comparable with birds dying on the farm. This convenient time interval was used to determine whether the migration of microörganisms took place within this period, rather than that they be considered an adequate control group for the project. The 7 birds, as well as the other 29 (cultured within one hour after being killed) failed to yield cultures.

DISCUSSION AND SUMMARY

Autopsies were made on 1,051 birds brought to the laboratory, dead or in a moribund condition. There were 274 or 26.1 per cent affected with reproductive disorders, compared with 777 or 73.9 per cent without these disturbances. The liver, heart blood, and spleen were routinely cultured. Isolations were made from 47.7 per cent of the birds. Cultures were obtained from 44.5 per cent of the birds with reproductive disorders, as compared with 48.8 per cent without the disorder.

Of the organisms isolated, 61.3 per cent were of the genus *Staphylococcus aureus* and *Staphylococcus albus*, and 26.7 per cent, *Escherichia coli*. The remaining 12 per cent included *Bacillus subtilis*, *Streptococcus viridans*, a hemolytic streptococcus, Salmonella spp., *Pasteurella avicida*, and a few unidentified species.

Some workers have associated the etiology of reproductive disorders with *P. avicida*, *Shigella gallinarum*, and *Salmonella pullorum* (1). It is quite evident that these pathogens were not concerned in the cases studied here. The flock has been pullorum disease-free for the past ten years, and fowl cholera and fowl typhoid were rarely found. Despite this fact, reproductive disorders occurred frequently in this flock.

There was little if any significant difference in the type or the incidence of bacteria isolated from birds with or without reproductive disorders.

Staphylococci and colon organisms repre-

TABLE 6—Results of the Inoculation of Birds with Pure Cultures of Bacteria

Type of Bacteria Inoculated	Number of Birds Inoculated	Dosage Used (c.c.)	Bacteria Reisolated From—	Condition of Birds Previous to Autopsy			
				Dead	Debilitated	Paralyzed	Normal
Sta. spp.	18	0.1 to 0.2	14 birds	3	3	0	12
Sta. spp.	7	0.3 to 0.4	7 birds	0	0	0	7
Sta. spp.	1	1.0	not cultured	1	0	0	0
Total	26		21 birds	4	3	0	19
E. coli	33	0.1	12 birds	5	6	12	10
E. coli	3	0.3	2 birds	0	0	1	2
E. coli	2	1.0	2 birds	1	0	0	1
Total	38		16 birds	6	6	13	13
P. avicida	6	0.2	3 birds	5	1	0	0
Salmonella spp.	1	0.1	0 birds	0	0	1	0
Grand Total	71		40 birds	15	10	14	32

sented 88 per cent of the organisms isolated from all birds cultured. Furthermore, these organisms were found in birds with and without the disease. No significant difference existed between the two groups of birds. Thus, it is concluded that Staphylococci and *E. coli* were of little or no etiologic significance.

The presence of these organisms might be explained on the basis that there is a violation of the normal barriers during the late or agonal stages of life. In other words, they may be secondary invaders, which develop as a result of reproductive disorders or some other debilitating condition.

A study of the pathogenicity of isolated organisms showed that 54.9 per cent were capable of causing illness or death in the dosage used. Thirteen cockerels, receiving inoculations with *E. coli* and one given Salmonella spp., developed a partial paralysis and general debility within a few days after being inoculated. Of the birds receiving *E. coli*, 34 per cent developed this condition. The symptoms and histopathologic findings were not pathognomonic of neurolymphomatosis.

Thirty-six apparently healthy yearling hens were cultured aseptically to determine the normal bacterial flora of birds. The organs cultured were the liver, heart blood, spleen, and oviduct. None of the hens yielded cultures of Staphylococci or *E. coli*.

ACKNOWLEDGMENT

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Acute Uremia Associated with "Uric Acid Infarcts" in the Kidneys of Baby Pigs

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AN UNUSUALLY large number of baby pigs died during the spring farrowing season of 1942 in the experimental swine herd at the Beltsville Research Center, Beltsville, Md. Reports of rather heavy losses among newborn pigs in some swine herds in the surrounding area were also received. The disease was observed again among spring and fall litters farrowed in 1943. Special attention was focused on the problem since the losses approached epizoötic proportions and the affected pigs exhibited symptoms which had not been noticed previously. Routine studies for the possible identification of the condition as an infectious disease were essentially negative. Spectrophotometric analyses* of the feed and water of the sows, and bones, liver, and urine of affected pigs failed to demonstrate toxic quantities of poisons such as lead, arsenic, mercury, or fluorine. No recent changes had been made in feeds or system of feeding,† and most of the sows were in excellent condition. In 1943, cases also occurred among baby pigs from sows fed three other types of rations, namely: all plant protein ration fed with corn and with wheat and a ration similar to the regular herd ration but containing a smaller proportion of tankage.

Several symptoms in affected animals appeared to be similar to those described by Graham, Sampson and Hester,[2] and Sampson and associates,[9,10] in so-called baby-pig disease, or hypoglycemia. Blood-sugar determinations on very sick pigs, however, often gave values within the normal range. A limited number of sick pigs hand fed with cow's milk containing 10 per cent of dextrose failed to show improvement. Intraperitoneal injections of 23 per cent calcium-gluconate solution, 40 per cent dextrose solution or thiamin did not produce any apparent clinical response, and pigs so treated usually died within twenty-four to forty-eight hours.

Autopsy findings were usually variable or negative except for the rather constant presence of a precipitate which could be most readily seen impacted in the papillary ducts of the kidneys (the so-called uric-acid infarcts) or lying free in the renal pelvis, ureters, or urinary bladder. The precipitate was usually of orange color, but occasionally it was nearly white. The finding of elevated nonprotein nitrogen values, along with the observations just mentioned, indicated impaired renal function. Further studies indicated that the elevation in nonprotein nitrogen was partially due to large increases above the normal level in blood urea and uric acid. Analyses of the precipitate found in the kidneys and on kidney tissue indicated that the precipitated material consisted largely of uric acid or urates. From these findings, it is believed that the cause of death in these baby pigs was associated with acute uremia.

It is the purpose of this paper to describe the symptoms, autopsy findings,

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The technical assistance of C. W. Thies and J. X. King is acknowledged.

*This phase of the work was carried out by Dr. Russell E. Davis and Harry Bastron.

†The herd grain ration consisted of a mixture of yellow corn and a protein-mineral supplement containing tankage 50 lb., linseed meal 25 lb., alfalfa-leaf meal 25 lb., and a complex mineral mixture, 5 lb. of which are added to each 100 lb. of protein supplement. The mineral mixture consists of ground limestone 50 lb., steamed bone meal 27.97 lb., salt 20 lb., iron oxide 2 lb., potassium iodide 0.02 lb. and copper sulfate 0.01 lb. The corn and supplement is mixed in the proportion of 7 parts of corn to 1 of protein-mineral supplement for gilts and 8 parts of corn to 1 of supplement for sows. The animals are hand fed daily an amount of grain ration equivalent to 2 per cent of their body weight for gilts and 1.5 per cent for sows. The animals are kept on pasture during the grazing season and during the winter they have free access to alfalfa hay in a rack.

blood chemistry and analyses of kidney tissues from cases of acute uremia and to describe initial attempts to produce the disease under controlled conditions.

MATERIAL AND METHODS

All baby pigs were observed daily for several weeks after birth. Sick pigs or suspected cases were noted and animals which had died overnight or had been accidently killed by the sow were collected for autopsy. Sick pigs which were treated were left either with the sow or removed to separate, heated pens if individual feeding was involved.

At autopsy, a wide field binocular microscope at 7 to 20 diameters was used to examine the kidneys for precipitate, although in the marked cases gross examination without magnification was satisfactory. Kidneys for histological examinations were fixed in acidified formalin solution‡ or absolute ethyl alcohol. The tissues were embedded in paraffin and stained with hematoxylin and eosin or the Schultz-Schmidt[12] method for uric acid and urates.

Blood samples for analysis were obtained by heart puncture. Blood-sugar determinations were made by the colorimetric ferricyanide method of Folin[8]. Determinations of urea nitrogen in whole blood were made by the urease method of Cullen and Van Slyke[8]. Determinations of uric acid in whole blood were made by the Benedict and Behre method[8], using a protein-free filtrate prepared without laking the blood. The Benedict and Behre method was also used in the determination of uric acid in extracts of kidney tissue. In the preparation of extracts of kidney tissue, one or both of the entire kidneys were finely ground by means of an Apolant tissue grinder. This ground tissue was received from the grinder into a weighing bottle in which it was stirred with a small spatula to insure adequate mixing before weighed samples of approximately 2 Gm. were transferred to 125 cc. Erlenmyer flasks. Sixteen cc. of distilled water and 4 cc. of M/15 borate buffer of pH 9.2 were added to each

TABLE 1—Diagnosis of Uremia Based on Presence of Orange-Colored Precipitate in the Kidneys of Baby Pigs from Spring and Fall Litters, 1943

Age of baby pigs	Spring pigs with kidney precipitate				Fall pigs with kidney precipitate			
	Dead pigs examined	Present	Daily incidence	Cumulative incidence	Dead pigs examined	Present	Daily incidence	Cumulative incidence
Days	Number	Number	Per cent	Per cent	Number	Number	Per cent	Per cent
1	35	6	17	17	67	12	18	18
2	14	5	36	22	18	8	44	24
3	10	7	70	31	17	14	82	33
4	11	9	82	39	9	8	89	38
5	12	10	83	45	7	7	100	42
6	12	7	58	47	5	4	80	43
7	3	3	100	49	11	7	64	45
8	5	2	40	48	4	3	75	46
9	4	4	100	50	3	1	33	45
10	5	0	0	48	4	0	0	44
Totals	111	53		48	145	64		44

‡4 drops concentrated hydrochloric acid to 5 cc. 36-38 per cent formaldehyde in 45 cc. water.

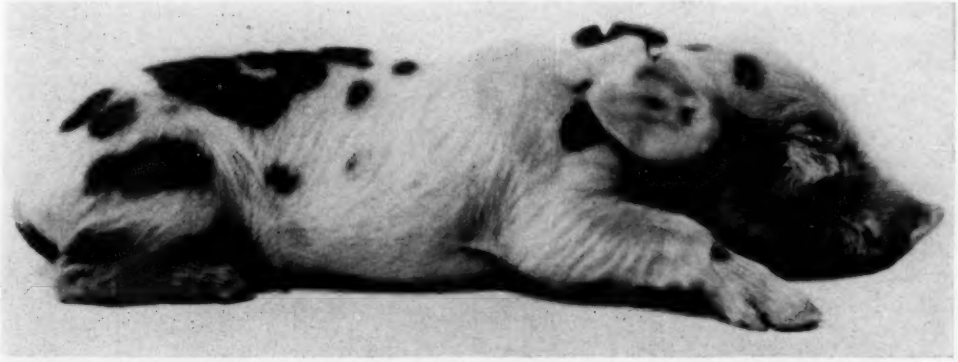

Fig. 1—This pig became moribund at 6 days of age after losing about 20 per cent of its weight from birth. When killed, considerable precipitate was found in the kidneys and urinary bladder. The blood contained 191 mg. of urea nitrogen and 18 mg. of uric acid per 100 cc. with kidney uric acid at 132 mg. per 100 Gm. of tissue. These findings are typical of severe uremia.

TABLE 2—Sugar, Urea Nitrogen and Uric Acid in the Blood and Uric Acid in the Kidneys of Normal Pigs, Suspected Cases and Affected Pigs

Conditions of pigs and amount of precipitate in kidneys	Age of pigs		Blood Analyses						Kidney Uric Acid	
			Sugar		Urea Nitrogen		Uric Acid			
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Days	Days	Mg./100 cc.	Mg./100 cc.	Mg./100 cc.	Mg./100 cc.	Mg./100 cc.	Mg./100 cc.	Mg./100 Gm.	Mg./100 Gm.
Normal pigs (no precipitate in kidneys)	5 (46)**	0-12	140 (36)	90-225	11.3 (41)	2.8-27.3	0.65 (20)	0.35-0.82	5.0 (10)	trace-11.6
Suspected cases (no precipitate in kidneys)	6.6 (7)	1-14	96.6 (1)	96.6	69.9 (6)	26.0-186.0	1.15 (6)	.7-1.82	13.5 (6)	5.7-30.0
Uremia cases (±* to + precipitate)	3.9 (11)	1-6	(2)	36-254	53.4 (8)	8.1-114.0	1.87 (6)	.72-6.0	9.6 (8)	trace -14.3
Uremia cases (+ + precipitate)	5.4 (16)	1-10	82.8 (7)	trace-220	125.1 (15)	3.9-335.0	5.0 (15)	.66-18.0	48.9 (12)	16.7-132
Uremia cases (+ + + to + + + + precipitate)	5.0 (13)	1-8	162 (4)	81-287	147.9 (10)	29.4-350	12.36 (6)	3.48-21.1	285.5 (11)	40-800

*The relative amount of precipitate in the kidneys was estimated on visual examination. The amount of precipitate seen is recorded ± which indicates a trace, + very slight amount, + + moderate, + + + large amount, and + + + + a very large amount of precipitate.

**All figures in parenthesis refer to the number of pigs on which analyses were made.

sample of tissue and the contents of the flasks were mixed thoroughly by shaking. After standing one hour at room temperature with occasional shaking, 1 to 2 cc. aliquots of the supernatant were removed for protein precipitation. The aliquots were diluted 1 to 10 with dilute tungstic acid protein-precipitating reagent and uric-acid determinations were made

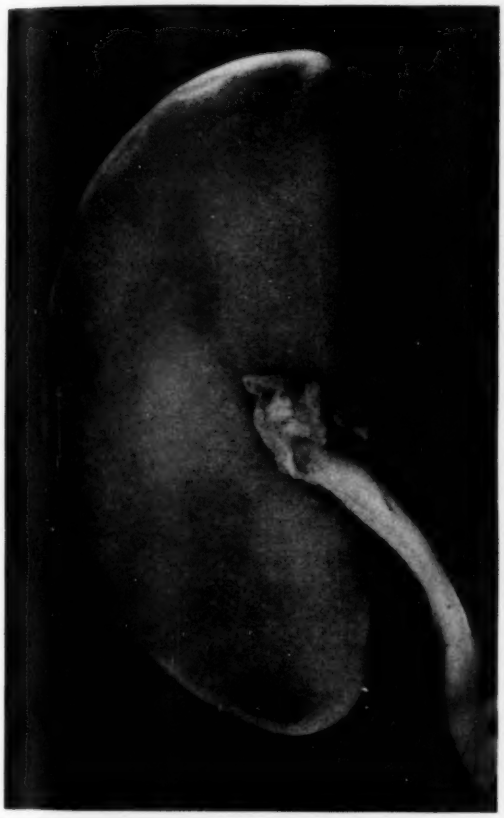

Fig. 2—Kidney showing accumulation of precipitated urates (small gray spots) in the cortical region of the kidney to the extent that they are visible on the surface.

on 5 to 10 cc. aliquots of the protein-free filtrate.

In all the cases on which data on the uric-acid content of the kidney tissue are included in table 2, the kidneys were removed from the animal within a few minutes after death. Kidneys were cut open longitudinally in order to make a visual inspection for the occurrence of precipitated urates and were then either ground immediately or placed in a covered weighing bottle in the ice box. Whenever possible, extracts were made on the same day that the animals were killed.

SYMPTOMS OF UREMIA

Affected pigs usually appear normal at birth but develop symptoms within one to three days after birth. They usually fail to gain in weight normally and often lose weight from birth. The hair coat becomes rough, the hair may stand on end, "goose flesh" is usually present and in later stages the body temperature becomes subnormal (fig. 1). The pigs may remain under the hover§ or isolate themselves about the pen. Diarrhea is fairly common in these cases, although this has also been seen in other young pigs that have remained apparently normal. For a few days, the pigs may continue to nurse but they gradually become weaker and go into coma. Some of the pigs appear extremely sensitive and will squeal feebly and have tremors when picked up or disturbed. Convulsions have been observed in advanced cases. The disease is highly fatal. Most of the pigs showing symptoms of this condition die or are accidentally crushed by the sow within the first nine days of life as indicated in table 1, which is discussed later. One or more members of a litter, or the entire litter, may succumb to the disease. The "runt" is often affected.

AUTOPSY FINDINGS

Gross changes noted at autopsy are quite variable except for the kidney findings mentioned previously. Typically affected animals are usually somewhat emaciated and dehydrated, although edema in the hind legs, inguinal region, along the abdomen and sternum, together with an excess of free fluid in the thoracic and abdominal cavities, are occasionally seen. The lungs and heart usually appear normal. The stomach and intestinal tract seldom show definite changes, although slight intestinal inflammation with evidence of diarrhea or impaction due apparently to constipation has been seen. The stomach is found to be empty or contains only a trace of curd in about 60 per cent of the cases. The liver is often apparently normal in color; in some

§In cold weather, heat is supplied for the baby pigs by means of an electric "pig-brooder," using a 150 watt bulb with a reflector.

cases, however, it is pale or yellowish with scattered areas of congestion. The bile is usually scanty and very concentrated, and often contains an orange-colored, flocculent precipitate.

The kidney capsule peels easily. The kidneys may be slightly pale or normal in color and they do not usually appear enlarged or swollen. Before removal of the capsule in a few very severe cases, close inspection has revealed an accumulation of precipitate in the cortex to the extent that it is visible on the surface of the kidney even through the capsule (fig. 2). If the kidney is pressed gently, the precipitate may be seen to flow in the ureter. Pressure on the kidney is not always necessary to demonstrate this because the amount of precipitate in the ureter may be so great that it is visible throughout its length. In severe cases, there is usually a collection of precipitate in the urinary bladder. In some cases, mechanical distention has apparently resulted because of the amount of precipitate present. In other cases, the bladder is either entirely empty of urine or contains a small amount of highly concentrated, turbid secretion suggestive of partial anuria.

Figure 3 illustrates the gross appearance of the impacted papillary ducts, together with an accumulation of precipitate in the pelvis of the kidney. One gains the impression from examining such kidneys that practically all of the papillary tubules may be blocked. Close examination often reveals some material deposited in the cortical region also. Varying amounts of the precipitate are seen on gross examination. In a few cases suspected of having uremia because of external symptoms and blood findings, the medullary region of the kidneys has been intensely congested but no precipitate has been seen. The relationship of this finding to the more common picture of impacted tubules without marked congestion or inflammation is not clear.

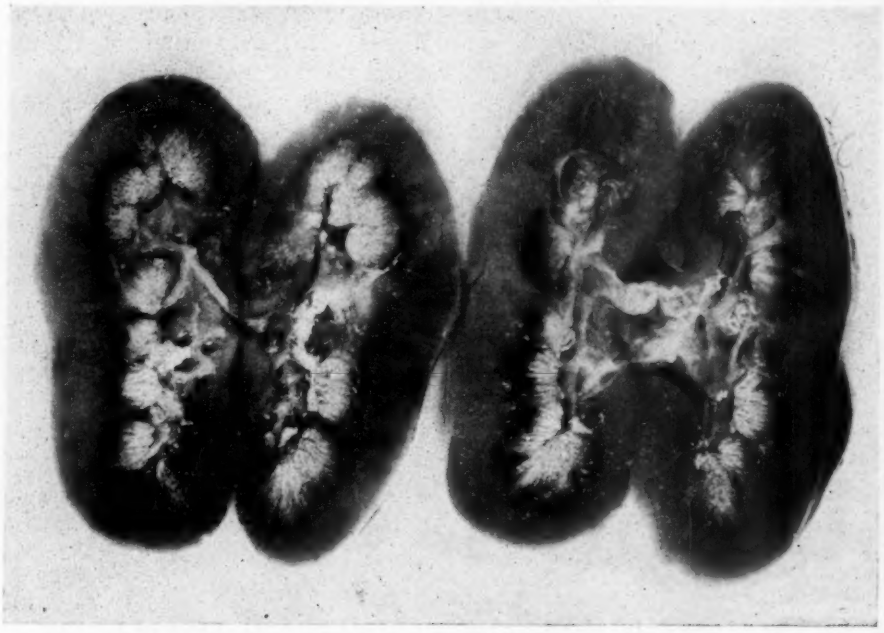

Fig. 3—Frontal sections of kidneys from a case of uremia showing heavy deposits of precipitated urates in the medullary substance particularly in the papillary region (the so-called uric-acid infarcts) and in the renal pelvis.

Figure 4 shows the papillary ducts distended with precipitate. In such cases, there may be extensive degeneration and

TABLE 3—Summary of Fasting and Limited Feeding Studies with Baby Pigs

Pig No.	Approximate age	Date	Food consumption	Estimated amount of kidney precipitate	Blood sugar	Blood urea nitrogen	Blood uric acid	Kidney uric acid	Remarks
			Experiment I						
	Hours				Mg./100 cc.	Mg./100 cc.	Mg./100 cc.	Mg./100Gm.	
2114	30	9/18	No food or water		133.8	9.8	0.72		
	78	9/20			80.0	30.8	1.28		
	90			+ + + +				365	Died during night, 9/20
2118	30	9/18	No food or water		115.9	9.8	0.61		
		9/19	No food or water						
	78	9/20	60 cc. water		70.0	35.0	1.00		
	90			+ + +				154	Died during night, 9/20
2119	30	9/18	No food or water		139.4	15.4	0.72		
		9/19	50 Gm. milk						
	78	9/20	50 Gm. milk		71.0	19.6	0.97		
		9/21	50 Gm. milk						
	126	9/22		0	96.1	53.2	0.40	9.9	Killed 9/22
2113	126	9/22	Nursing sow	0	146.7	10.5	0.35	11.6	Normal litter mate control
			Experiment II						
2194	16	9/28	No food or water		123.7	11.2	0.48		
	64	9/30			98.0	49.0	0.40		
	112	10/2		0	96.6	81.2	0.70	5.7	Killed 10/2
2195	16	9/28	No food or water		126.2	8.4	0.52		
	112	10/2		+	93.4	131.6	0.87	8.8	Killed 10/2
2193	16	9/28	75 Gm. 20% glucose daily		132.0	8.4	0.51		
	112	10/2		+	73.9	91.0	1.19	12.0	Killed 10/2
2192	16	9/28	75 Gm. milk daily		126.2	14.0	0.48		
		9/30	1 mg. thiamin added						
	112	10/2		+ +	139.4	176.4	1.07	24.8	Killed 10/2
2191	16	9/28	Nursing sow		167.8	9.8	0.70		Normal
	184	10/5	Nursing sow	0	220.0	11.2	0.82	2.3	litter
2196	184	10/5	Nursing sow	0	225.0	11.2	0.82	6.9	mate
2197	184	10/5	Nursing sow	0	194.1	11.2	0.73	6.6	controls
			Experiment III						
2201	18	9/28	No food or water		162.3	14.0	0.56		
	114	10/2		+ +	108.8	81.20	0.73	23.9	Killed 10/2 all litter mates normal

exfoliation of the tubular epithelium as shown in figure 5. Kidney sections stained by the Schultz-Schmidt method reveal that the crystalline material in the tubules gives color reactions characteristic of monosodium urate and uric acid.

UREMIA BASED ON KIDNEY EXAMINATION

Most of the baby pigs from the spring and fall litters of 1943, which died up to 30 days of age, including stillborn animals,* were examined *post mortem*. In all, 380 animals were examined, including 64 stillborn pigs. A diagnosis of uremia was recorded when precipitate was found in the papillary ducts or pelvis of the kidney.

We have never detected precipitate in the kidneys of apparently normal pigs which have been killed for examination or which were accidentally killed by the sow. A summary of these data on animals dying within the first ten days of life is given in table 1. The condition was found to be rare in stillborn animals, since only 2 cases from a single litter were found. Tabulation of data on pigs dying between the ages of 11 and 30 days are intentionally omitted from the table, because the incidence of the disease among animals of this age group is low. In 69 pigs examined, which were older than 9 days, only 6 cases with kidney precipitate were found, and the oldest animal affected died when 17 days old.

It is generally known that the highest death loss among baby pigs occurs on the first day of life. This is well illustrated in table 1. However, the incidence of uremia in the 1943 spring and fall pigs on the first day of life was only 17 and 18 per cent of the total death, respectively. The incidence of uremia increased rapidly after the first day, and by the fifth day practically all of the pigs examined had varying amounts of precipitate in the kidneys which was usually associated with typical symptoms before death, as previously described. The cumulative incidence of uremia (percentage of all dead pigs showing deposits in kidneys) reached a maximum of 50 per cent on the ninth day among the spring pigs, and 46 per cent on the eighth day in the fall pigs. Accidental deaths due to the baby pigs being stepped on or mashed by the sow on lying down was the principal cause of death in pigs which did not show gross evidence of uremia. Twenty-nine of the 123 cases of uremia found in the spring and fall litters also showed evidence of accidental injury. In some cases, the injury was severe enough to have been the immediate cause or a contributing factor in the death of the animals.

*This group includes pigs which may have been alive at birth but died shortly after without evidence of lung inflation.

CHEMICAL STUDIES

When sickness among baby pigs was first observed in the spring of 1942, symptoms exhibited suggested hypoglycemia as previously pointed out, but blood-sugar determinations on some very sick pigs did not appear to substantiate this view, although a few cases with low blood sugar were found. Serum calcium and inorganic phosphorus were found to be within the normal range in both affected pigs and their mothers. Blood magnesium tended to be high in sick pigs. Values for blood magnesium among normal pigs of this age group were found to vary between 1.90 and 2.75 mg. per 100 cc. of blood, but values for affected pigs ranged between 2.71 mg. and 4.76 mg. With the discovery of elevated nonprotein nitrogen in the blood of affected pigs, interest shifted to the urea and uric-acid levels, since we regarded these findings as significant in view of the symptoms and autopsy findings observed. Kidneys have also been analyzed for uric-acid content, and in recent studies a few additional blood-sugar determinations were made.

In table 2, blood sugar, urea nitrogen, uric acid, and kidney uric-acid values are listed for normal pigs, suspected cases of uremia in which no kidney precipitate was observed, and for cases of uremia in which varying amounts of kidney precipitate were found.

Fewer data are listed in the table for sugar than for other blood constituents studied, since only the recent data on cases where the estimated amount of precipitate

has been recorded are included. An average value of 140 mg. of sugar per 100 cc. of blood was found in 36 analyses on normal pigs. Several cases of low blood sugar are recorded, but the average and range for the most severe uremia cases (+++ and ++++) were within the normal range or above. Low blood sugar appears to be associated with a markedly subnormal body temperature in the terminal stages of the disease.

Urea-nitrogen values are interesting when considered in relation to increasing severity of the symptoms of uremia. An average of 11.3 mg. of urea nitrogen per 100 cc. of blood was found in 41 normal pigs. In general, pigs having the clinical appearances of the condition under study tended to show a high level of urea nitrogen in the blood. Five of the 6 cases in which no precipitate was found at autopsy were found to have high urea nitrogen. Three of the 8 pigs graded ± and + cases had urea-nitrogen values within the normal range, although the others were well above normal. One of the 15 ++ cases, in which urea-nitrogen determinations were made, also had a low value although the average for the group is greatly elevated. The +++ and ++++ cases always had urea-nitrogen values above the normal range and the highest value of 350 mg. per 100 cc. is approximately 30 times greater than the normal average.

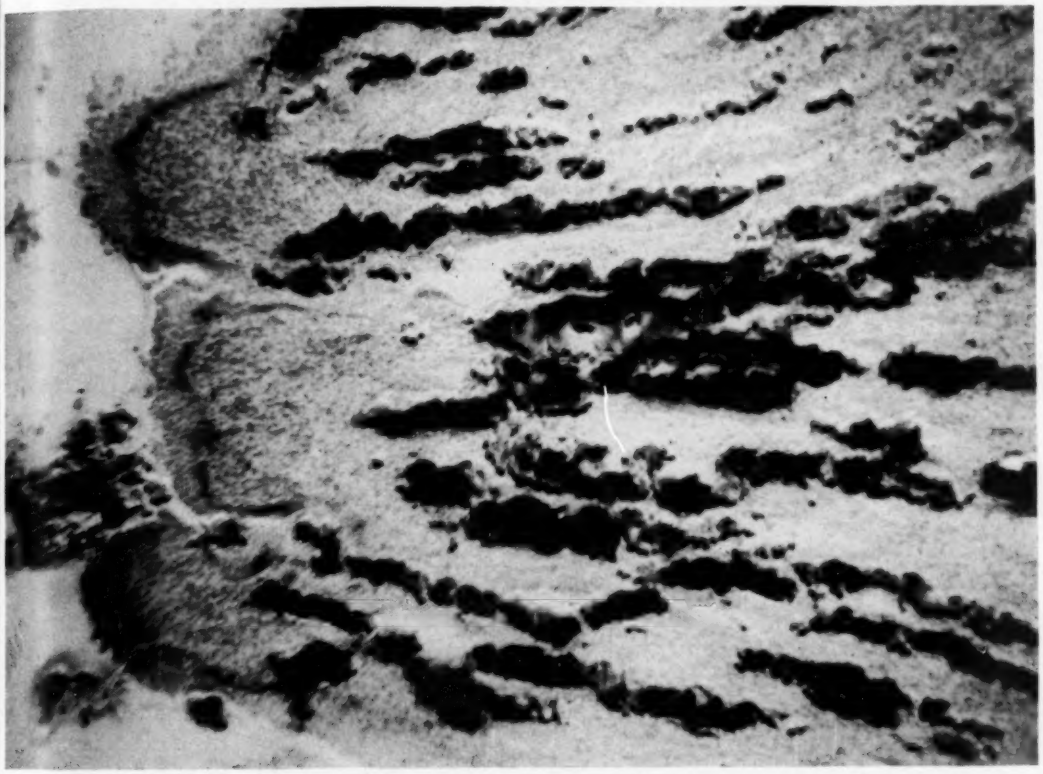

Fig. 4—Longitudinal section of a kidney papilla showing deposits of precipitated urates in the large collecting tubules and papillary ducts. X 90.

The uric-acid levels in the blood of affected pigs varied in a manner similar to that of the urea levels. The average value for the cases showing clinical symptoms without precipitates in the kidneys was above the normal range, although a normal level was observed in some individuals. The average blood uric acid increased slightly in the ± and + cases and was distinctly higher in the ++ cases, but a few animals

had values within the normal range in those groups. In the severe cases, blood uric acid was consistently elevated, being more than 30 times normal in some individuals.

The amounts of uric acid extracted from kidney tissue of 10 normal animals ranged from a trace to 11.6 mg. per 100 Gm. of tissue. It was estimated that a value of 5 mg. per 100 Gm. of tissue represents an approximate average for the normal pigs. The average of values obtained on affected pigs with clinical symptoms but with no visible precipitate in the kidney tubules (suspected cases) was above the range for normal animals, although values obtained on some individuals in this group, as well as on some cases in the group which showed a very small amount of precipitate in the kidneys, fell within the range of so-called normal values. In the most severe cases, an average of 285.5 mg. of uric acid was found with a maximum of 800 mg. per 100 Gm. of kidney tissue in one case. This represents approximately a 57- to 160-fold increase in uric-acid content above the normal kidney average, respectively.

While the range in individual values was rather wide in numerous cases, the analytical results on the whole show that as the amount of visible precipitate in the kidneys increased the blood-urea and uric-acid levels and amount of uric acid in the kidney tissue also tend to increase.

Some preliminary observations indicate that the uricolytic activity of the kidney tissue of affected pigs may be impaired.

STUDIES ON THE CAUSE OF UREMIA

Since early attempts to demonstrate that the disease was infectious or due to ordinary poisons were essentially negative, work is being continued on the problem with special attention given to the diet of the sow in relation to the incidence of the disease, together with observations on factors possibly related to the deranged metabolism of affected pigs. Some preliminary findings on the latter phase of the work are available at this time.

Attention has been centered on the possibility of partial or complete starvation in the early hours of life as a factor in the disease, since the stomachs of affected pigs are often empty or contain only a small amount of curd. This is not always true, however. Starvation is also suggested by the fact that affected pigs usually fail to gain normally and in advanced stages usually appear thin and dehydrated. Direct evidence suggesting inanition has also been obtained by weighing pigs before and after nursing from several sows that had a high proportion of affected pigs. In most cases, the affected animals did not get sufficient milk to maintain themselves, even though they nursed vigorously in the early stages of the disease.

In preliminary studies on the effects of starvation, 12 young pigs from 3 apparently normal litters were subjected to various treatments, and analyses made on blood and kidney tissue. The results obtained with each litter are summarized separately and are listed as experiments I, II, and III in table 3.

In experiment I, 3 young pigs approximately thirty hours after birth were taken from the sow. The plan was to starve 1 pig completely, give 1 water, and the third a limited amount of milk. A fourth pig was left with the sow as a normal control. Blood samples were taken before the experiment started, when the pigs were about 78 hours' old, and again at 126 hours from the survivor on limited milk, and from the control. Pig 2114 died when about 90 hours old. The kidneys were heavily loaded with precipitate. At 78 hours' old, the blood sugar was at a fasting level but not low. The blood-urea nitrogen was elevated and uric acid had increased slightly. The kidneys were found to contain 365 mg. of uric acid per 100 Gm. Pig 2118 refused water until the second day and died at about 90 hours old, showing much the same condition as pig 2114. Pig 2119 on limited milk survived but was killed for examination at about 126 hours after birth. In this case, no precipitate was seen in the kidneys but the blood-urea nitrogen was higher than normal. Blood and kidney uric acid were within the normal range. The control pig was found to be normal in all respects.

In experiment II, 7 pigs were used. Two

pigs were given no food or water, one was given 75 Gm. of 20 per cent glucose solution per day and another 75 Gm. of milk, with 1 mg. of thiamin added per day after the second day. Three control pigs were allowed to suckle normally. The pigs were killed for examination at about 112 hours of age.

litter of 5 was deprived of food and water commencing eighteen hours after birth until 114 hours of age when it was killed for examination. A moderate amount of precipitate was found in the kidneys, blood sugar remained normal, urea nitrogen was distinctly elevated in the blood and an in-

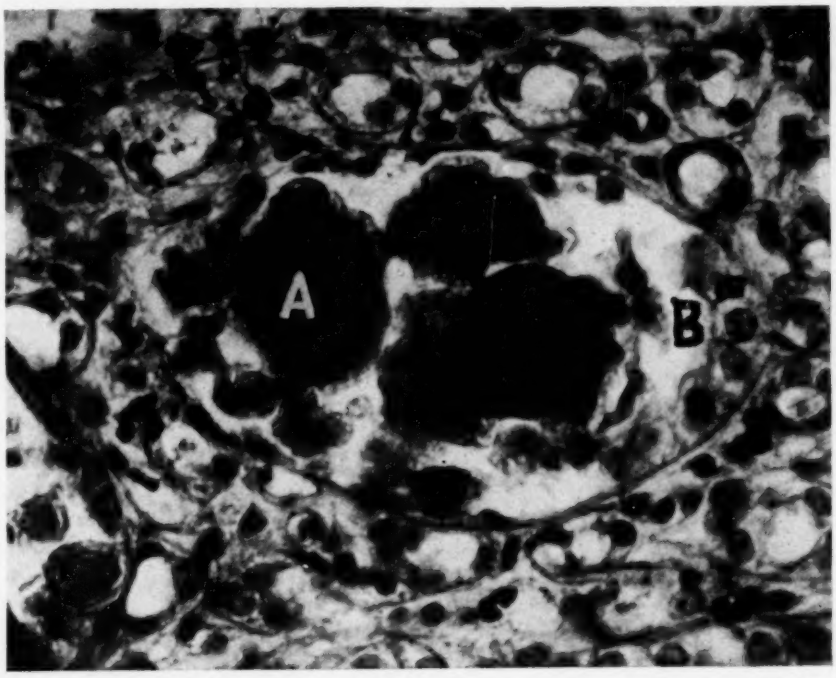

Fig. 5—Cross section of a collecting tubule containing precipitated urates, A. Note four or five apparently normal cuboidal epithelial cells at B with degeneration and extensive exfoliation of the epithelium around the remainder of the tubule. X 600.

A small amount of precipitate was found in the kidneys of pigs 2195 which received no food, 2193 which was fed glucose, and 2192 which was fed a limited amount of milk plus thiamin. The blood sugar remained apparently normal in all cases, but the blood-urea nitrogen was markedly elevated in all but the controls. Blood uric acid was slightly elevated in 2 pigs which also showed small amounts of precipitate in the kidneys and a slight increase in uric acid in kidney tissue. In the other 2 fasted pigs, there was marked elevation in blood urea but the blood and kidney uric acid remained within the normal range. The controls remained apparently normal.

In experiment III, a single pig from a

creased amount of uric acid in the kidneys was found.

These experiments were consistent in showing that a condition very similar to uremia as described in baby pigs can apparently be produced by withholding food from young, apparently normal animals, or by limited feeding on milk or glucose solution.

DISCUSSION

There is considerable evidence that the condition we have described in baby pigs may have its counterpart in human infants.[14,11] It is well known also that the blood uric acid and uric-acid excretion of the newborn is high,[4,11] and that there is

a similar trend in the blood nonprotein nitrogen.[13] With reference to human beings, Schloss and Crawford[11] state that, "During the first few days in the life of an infant a number of phenomena may occur which border closely on the pathologic, among which is the frequent, if not constant, deposit of uric acid or its salts in the kidney tubules." They continue by saying, . . . "in a large percentage of the infants who die during the first few days of life, a deposit of uric acid or urates is found in the medullary portion of the kidney—the so-called uric acid infarcts. It has been observed that these deposits are rarely present in dead-born infants; that they are found with greatest frequency in infants dying between the second and eighth days of life, and that their occurrence is infrequent after the twentieth day."

The parallelism between the condition found in human infants and the condition described in baby pigs seems more than a coincidence. According to information received by one of the authors (L. L. M.)‡, the condition described in human infants is now rather rare if a relatively high fluid intake is established early in life so as to stimulate kidney function. The application of this preventive therapy to the problem in baby pigs has some practical difficulties, but factors favoring a prompt and adequate milk flow are suggestive of having some bearing on the problem, particularly in view of our preliminary results on the effect of starvation.

Fasting is known to influence the concentration of nonprotein nitrogenous compounds in the blood of the animals as well as the pig, as shown in this study. Bang[1] found an increase in the nonprotein nitrogen, (n.p.n.) in the blood of fasting rabbits. Morgulis and Edwards,[7] working with dogs during fasting and refeeding, found an increase in blood n.p.n. and urea in the early stages of fasting with a greater increase toward the end of a prolonged fast. They also report that uric acid increased progressively during fasting and that inanition without water intensified these blood findings. Refeeding promptly shifted the blood picture towards normal. Similar results have been observed in fasting human beings.[5,3]. Lennox[5] noted decreased excretion as a factor in increased uric-acid retention during fasting but this was not paralleled by increases in blood n.p.n. or urea nitrogen when the kidneys were normal.

It is apparent that the chemical findings on the blood and kidneys of affected pigs, particularly with regard to urea and uric acid, are adequate to explain the clinical symptoms and autopsy findings. The possible relationship of uremia in baby pigs with the so-called baby-pig disease or hypoglycemia is not clear since data on the nonprotein nitrogen fractions of the blood were not included in the available reports on hypoglycemia.[2,9,10] From the data we have available, it appears that hypoglycemia is not an essential feature of typical uremia, although cases with low blood sugar have been observed. This is not surprising in view of the experimental findings on the possible rôle of fasting in the production of uremia and the known susceptibility of newborn pigs, along with the refractory nature of older pigs to fasting hypoglycemia.[9]

Our preliminary findings which suggest that the uricolytic activity of the kidneys of affected pigs is low as compared to normal animals recalls the early work of Mendel and Mitchell,[6] who were unable to demonstrate uricolytic enzyme (uricase) in embryo pig tissues. They conclude that the enzyme appears shortly before or after birth. Factors inhibiting uricolytic activity of the tissues and other conditions such as dehydration and acidosis may also be involved in the production of this condition in baby pigs. These possibilities are being investigated further.

SUMMARY

Acute uremia has been diagnosed among baby pigs dying during the 1942 and 1943 farrowing season at the Beltsville Research Center. Evidence is presented which indicates that 45 to 50 per cent of the baby pigs dying within the first nine days of life

‡Personal interview with Dr. P. György.

during the spring and fall farrowing season of 1943 were affected with this condition. Typical cases of uremia have a considerable amount of orange-colored precipitate in the kidneys, ureters and urinary bladder; such cases show a marked elevation in blood urea nitrogen and uric acid and a marked increase in uric acid in the kidney tissue. Blood sugar may be within the normal range or much lower than normal. Withholding food entirely or limited feeding of baby pigs in preliminary work has resulted in the production of a condition that is very similar to acute uremia as encountered under herd conditions.

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Symptoms Caused by Experimentally Produced Lesions in the Cerebrum of Dogs

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SPONTANEOUS lesions are of frequent occurrence in the cerebrum of dogs and various symptoms have been attributed to their presence. However, the causes of these lesions are various and one rarely knows how long a lesion has been present before symptoms appear; therefore, their significance often is doubtful. We were interested in the relation of certain focal lesions in the cerebrum to the occurrence of recurrent epileptiform fits and convulsions in dogs. We attempted to produce similar symptoms experimentally by making specific lesions in the cerebrums of normal dogs. The lesions were produced surgically. The same methods were used in all instances. The method employed and the results obtained will be discussed.

Various methods for the experimental production of lesions in the brain of animals have been described in the literature. We used the method employed by Speransky,[1] who made sterile lesions in the brain of animals by freezing certain selected regions.

METHODS

The dorsolateral surface of the cerebrum was the site selected by us for the production of lesions. Our surgical procedures were as follows:

The dogs were anesthetized with ether and the surgical field was carefully prepared. The skin, fascia, and muscles were incised along the parietal and frontal crest and were reflected laterally, exposing the parietal and frontal bones. An opening was then made through the skull with a trephine over the site selected for a lesion. This opening extended through the skull only and did not penetrate the dura. A piece of carbon-dioxide snow approximately 2.5 cm. in diameter was then placed directly on the dura and was held in place for two minutes. We had learned previously that freezing the brain for one minute would not produce definite lesions in every instance. Immediately after completion of the freezing, the opening in the skull was closed by replacing the piece of bone removed by the trephine. This was merely laid in the opening and the muscles, fascia, and skin were closed in layers over it. Immediately after closure of the wound, the dogs were permitted to recover from the anesthetic and were carefully observed for the first appearance of symptoms.

Lesions were made in various areas of the cerebrum. Each lesion was 2 cm. or more in diameter. In some instances, the lesions were on or near the cruciate fissure and were definitely in the motor area. However, in most instances, they were 2 cm. or more posterior to the cruciate fissure and extended laterally to the ectosylvian or ectolateral fissure. The motor activity of each area was determined by direct faradic stimulation immediately prior to freezing. At the time of the first operation, lesions were made in the left cerebral hemisphere and the dogs that recovered were operated on again after an interval of twenty-one to eighty days. At this time, a lesion was made in the right cerebral hemisphere. The same surgical procedures and methods were used in each instance.

RESULTS

For convenience, the results will be discussed under two headings, series 1 and 2. In series 1 the results obtained after the first operation will be discussed, and series 2 will deal with the results noted after the second operation.

Series 1.—Sterile lesions were produced experimentally in the left cerebral hemisphere of 20 dogs. Eight (40 per cent) of these animals manifested nervous symptoms which could be attributed to the lesion produced in the cerebrum and 12 (60 per cent) remained entirely free of nervous symptoms (table 1). The symptoms noted consisted chiefly of convulsions and epilep-

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tiform fits, which were graded 1 to 4 according to their severity and duration. These symptoms developed forty minutes to seven hours and thirty minutes after the lesion was made and were transient. Two of these animals, dogs 12 and 18, had subdural hematomas at the site of the lesion

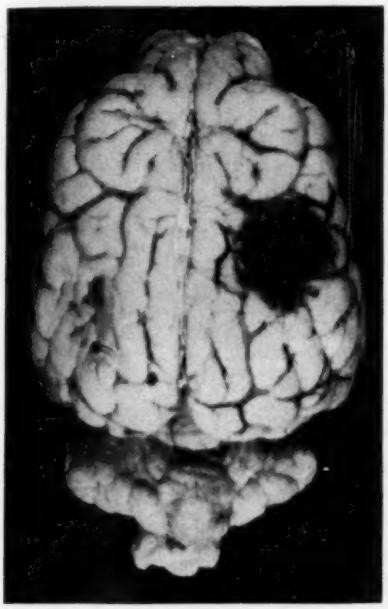

Fig. 1—Two lesions are present. The one in the left hemisphere has healed and only a shallow depression marks the site. The lesion in the right hemisphere is healing. It is well circumscribed. Both lesions caused epileptiform fits. The operative wound had healed and the animal was apparently normal on the tenth postoperative day when it was anesthetized and destroyed so that the lesions could be obtained for study.

and succumbed; the remaining 6 that had nervous symptoms made a complete recovery. Dogs 5 and 15 will be discussed individually, whereas dogs 1, 8, 10, and 13 will be grouped for discussion.

In dog 5, an epileptiform fit developed forty-five minutes after operation. The fit was initiated by localized convulsions which rapidly became general. During the recovery period, the dog showed a tendency to run and bark, a typical running-barking fit. Several similar but less severe attacks were observed during the following hour, after which they ceased to occur. The animal was apparently normal on the day following the operation and remained so until after a second lesion was produced thirty-five days later.

In dog 15, a mild epileptiform fit developed forty minutes after operation. This fit was of short duration but during the succeeding five hours, several similar but more severe attacks were observed. They were of two to four and a half minutes' duration. Salivation, twitching of the muscles of the head, and jerking movements of the right foreleg were the first symptoms noted, but as each fit progressed the convulsion became general. Champing of the jaws and running movements were observed during the recovery period. The symptoms were similar to, but less severe than, those manifested by dog 5.

In each of dogs 1, 10, and 13, one mild epileptiform fit developed, and in dog 8, two

Fig. 2—Cross section of an acute hemorrhagic lesion. A subdural hematoma had developed and the animal had succumbed seven hours after operation.

similar attacks developed. They occurred four to seven hours after operation and were of short duration. The symptoms noted during each fit were twitching of the muscles of the face, clonic jerking of the legs, champing of the jaws, and salivation. Jerking of the legs was most evident on the right side and running movements were manifested during the recovery periods in

all instances. Each fit began as a localized convulsion which tended to progress rapidly and become general. All of these dogs were apparently normal on the day following the operation.

Series 2.—After an interval of twenty-one to eighty days, a lesion was made in the cerebrum in the 18 dogs that had recovered after the first operation. The second lesion was made in the right cerebral hemisphere so that each animal now had bilateral lesions in the cerebrum.

The occurrence of symptoms was more frequent after the second operation than after the first; in 15 cases (83 per cent), convulsions and epileptiform fits now developed, whereas, in only 40 per cent had these symptoms been manifested after the first operation. The fits were severe in 12 cases, and in 5 cases extensive hemorrhage developed at the site of the lesion and the animal died.

The symptoms manifested were in most instances similar to those noted after the first operation, the only difference being that the fits were thought to be more severe and the clonic jerking of the legs was more disorganized. The postoperative time of occurrence of the symptoms was varied. In two cases, the symptoms appeared as early as one hour after operation but in another case they did not occur until eleven hours and thirty-two minutes after operation. None of the dogs that recovered manifested symptoms after the first postoperative day. Since the symptoms observed were like those that had occurred after the first operation, they will not be discussed in detail.

The depth of the lesion and the occurrence of subdural hemorrhage appeared to determine the severity of the symptoms manifested, it was observed that the lesions were deep and subdural hemorrhage was present around them in all of the animals that died after operation. But since we could not simultaneously compare these lesions with those present in the animals that recovered, this can be presented as suggestive evidence only.

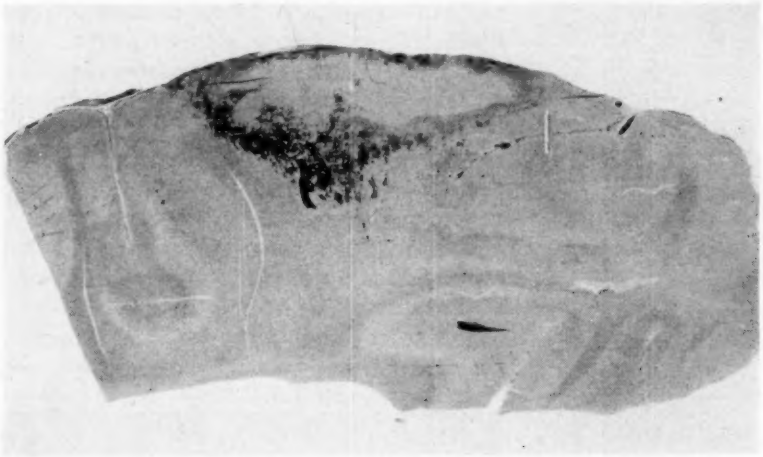

Fig. 3—Cross section of a healing lesion of the cerebrum. The lesion is 30 days old. Healing is well advanced. The dark area is hemorrhage and masses of phagocytic cells which are there removing the tissue debris.

COMMENT

Epileptiform fits were the chief symptoms noted after experimental production of lesions in the cerebrums of dogs. In most instances, the fits began as localized convulsions, which rapidly spread and became generalized. The symptoms noted during each attack were characterized by twitching of the muscles of the face, clonic jerking of the legs, champing of the jaws, and salivation. Running movements were common during the recovery period. Disturbances of intelligence and sight were not observed. The symptoms in general were similar to those noted in dogs affected with various spontaneous diseases of the

cerebrum. One dog showed a tendency to run and bark when recovering from a fit. However, it is interesting to note that the symptoms produced by the lesions made in the cerebrum did not persist. They were not observed after the first postoperative day in the dogs that recovered. When unilateral lesions were present, the convulsions manifested indicated the hemisphere of the cerebrum that was affected but did not permit further localization of the lesion. Symptoms were not manifested by all of the dogs in which lesions were made. This fact could not be attributed to the site of the lesion, because lesions in the same site in other dogs caused severe symptoms. Lesions in the motor area always caused convulsions, which were sometimes localized, but similar lesions 2 cm. or more removed from this area often caused like symptoms. It is possible that the depth of the lesion was the deciding factor.

Fulton[2] stated that the physiologic function of the temporal lobes in animals is not known but that Marquis' work indicates that bilateral removal of the occipital lobes of dogs affects their vision. We did not observe disturbances of vision in the dogs in which we made lesions in the occipital lobes.

Speransky expressed the opinion that the symptoms manifested after freezing of small regions of the cerebrum were due to dispersion of toxic products which occur in the lesion. He stated that animals acquire resistance to these toxic substances. If this is true, the effects of a second lesion should be less severe than those following production of the first lesion. Our experiments did not confirm Speransky's opinion.

The wounds in the skull and brain healed rapidly. The skull appeared completely healed within three weeks. The piece of bone removed by the trephine, although entirely detached from the skull during operation, healed in place and was smooth. The dura usually adhered to the cerebral lesion as well as to the lesion in the skull, but this apparently did not cause any ill effects. Frequently only a depressed area marked the site of the cerebral lesion (fig. 1). The lesion did not contract into a puckered scar and therefore did not cause distant effects by pulling on the vaso-astral framework of the brain, a condition mentioned by Penfield.[3] Figure 2 shows an acute hemorrhagic lesion, which caused the death of the animal.

Microscopic examination of the healing process in the cerebrum revealed a reaction somewhat like that caused by infectious agents. The large nerve cells necrosed and disappeared and leucocytes occurred in clumps at the margin of the lesion (fig. 3). The segmented forms seemed to be more numerous than in lesions caused by neurotropic viruses. The leucocytes frequently were massed in nests but they did not collect in the perivascular spaces as they do

TABLE 1—Effects of Lesions in the Cerebrum of Dogs

	First operation			Second operation			
	Convulsion			Convulsion			
Dog	Grade	Postoperative time	Result	Time*	Grade	Postoperative time	Result
1	3	6 hr. 5 min.	Recovered	80	4	1 hr.	Died
2	—		Recovered	42	4		Recovered
3	—		Recovered	55	2	10 hr.	Recovered
4	—		Recovered	28	1	6 hr. 15 min.	Recovered
5	4	45 min.	Recovered	35	4	1 hr. 20 min.	Recovered
6	—		Recovered	28	—		Recovered
7	—		Recovered	28	—		Recovered
8	2	5 hr. 35 min.	Recovered	28	4	1 hr.	Died
9	—		Recovered	28	—		Recovered
10	1	4 hr.	Recovered	49	4	2 hr. 15 min.	Died
11	—		Recovered	49	2	1 hr. 45 min.	Died
12	1	6 hr. 30 min.	Died				
13	2	7 hr. 30 min.	Recovered	48	4	5 hr.	Recovered
14	—		Recovered	48	4	7 hr. 50 min.	Died
15	4	40 min.	Recovered	27	4	1 hr. 30 min.	Recovered
16	—		Recovered	27	4	4 hr. 37 min.	Recovered
17	—		Recovered	27	2-4	4 hr. 52 min.	Recovered
18	4	45 min.	Died				
19	—		Recovered	21	4	11 hr. 32 min.	Recovered
20	—		Recovered	21	4	5 hr. 27 min.	Recovered

*Interval before second operation, days.

in lesions produced by infectious agents. The glial elements proliferated throughout the lesion and the slough was removed by phagocytic cells. A minimum of distortion of the surrounding tissues was observed. In some instances, the gray matter had entirely disappeared from the site of the lesion. The healed lesion (fig. 4) closely resembled one resulting from surgical excision of a portion of the brain.

Fig. 4—A healed lesion of the cerebrum. Note that nothing but some scar tissue remains. This view was obtained on the sixty-second postoperative day.

Dogs frequently receive traumatic wounds of the skull in automobile accidents, falls, and so forth, which cause varying degrees of injury to the brain. When such a case is presented to a veterinarian, the owner often inquires: "Will this dog be all right if it lives?" One cannot, of course, give definite assurance of this, but the results of these experiments indicate that dogs can recover and not experience any apparent ill effects when lesions as large as 3 cm. in diameter are made in the cerebrum.

SUMMARY

Lesions were made in the cerebrums of two series of dogs by freezing selected areas with carbon-dioxide snow. They were unilateral in one series and bilateral in the other.

Epileptiform fits were the chief symptoms noted after operation. They occurred in 40 per cent of the dogs having unilateral lesions and in 83 per cent of the dogs that had bilateral lesions. The epileptiform fits were characterized by twitching of the muscles of the face, champing of the jaws, salivation, and clonic jerking of the legs. One dog showed a tendency to run and bark during the recovery period. Epileptiform fits and convulsions did not occur longer than twenty-four hours after operation in the animals that survived.

The site and depth of the lesion appeared to determine the character of symptoms. Lesions in the motor area always caused local and general disturbances but lesions 2 cm. or more posterior to this area usually caused similar symptoms.

The lesions healed rapidly and only a shallow depression remained after six weeks to mark the previous site of the lesion.

Microscopic examination of the lesions during the healing process revealed hemorrhage and infiltration of leucocytes. Segmented forms of leucocytes predominated. They frequently were massed in clumps but they did not collect in the perivascular spaces as they do in lesions produced by infectious agents.

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A Salmonella Choleraesuis Bacteriophage in Swine Feces

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ALTHOUGH various bacteriophages have been found in the feces of different animals and man, we have not encountered any report in the literature of the presence of a bacteriophage for *Salmonella choleraesuis* in the feces of swine. In 1926, the Bureau[1] reported that results of efforts to determine whether or not a bacteriophage was present in intestinal contents of swine were entirely negative. Levine and Frisch[2] reported the results of their work with a number of bacteriophages affecting different members of the *S. choleraesuis* group. All of the bacteriophages with which they worked were recovered from the feces of chickens.

There have been numerous reports of bacteriologic examinations of the feces of swine for the presence of *S. choleraesuis*, particularly the feces of normal swine. The incidence of recovery of this organism has usually been 3 to 4 per cent. In none of the reports that we have reviewed has there been mentioned the possibility of the presence of bacteriophage being a factor responsible for the negative findings.

EXPERIMENTAL

In a series of experiments, bacteriologic examinations of swine feces were made for the presence of Salmonella, with particular reference to *S. choleraesuis*. The feces from 134 pigs were examined. Of this number, 73 were exposed by feeding broth cultures of *S. choleraesuis* var. *kunzendorf*. This organism was obtained from the feces of only 5 of the exposed pigs and from the feces of only 3 of the unexposed. In the case of the exposed pigs, the examinations were made from one to five weeks after exposure. *S. choleraesuis* var. *kunzendorf* was recovered from one or more tissues (heart blood, spleen, kidney, liver, and gall bladder) of 29 of the exposed pigs which included the 5 from which it was obtained from the feces. It was obtained from the tissues of 6 of the unexposed pigs which included the 3 from which this organism was obtained from the feces. Numerous types of Salmonella other than *choleraesuis* were obtained. Failure to recover *S. choleraesuis* from the feces of more of the exposed pigs was thought at first to be due to the type of medium used or to some inhibitory action of other bacteria. Endo's, E.M.B., bismuth sulfite, SS, and brilliant green agars, tetrathionate broth and Ruy's medium were used.

When a test for bacteriophage first was made, a mixture of 2 or 3 Gm. of feces from 3 pigs was seeded in 25 cc. of beef broth, incubated overnight at 37 C. and filtered through a Berkefeld N candle. The bacteria-free filtrate was then tested for its inhibitory action against four types of Salmonella, namely, *choleraesuis* var. *kunzendorf*, *derby senftenberg*, and *oranienberg*. One cc. of the filtrate was added to tubes containing approximately 12 cc. of beef broth. Four tubes of this medium were seeded with a 1-mm. loopful of 24-hour broth culture of each of the four types of Salmonella, respectively. Tubes of broth without filtrate also were seeded for controls. After overnight incubation at 37 C., there was no visible growth in the tube containing filtrate and seeded with *S. choleraesuis* but the usual heavy growth was in the control tube. There was no difference in the density of growth in the filtrate and control tubes seeded with *S. derby* and *S. senftenberg*. There was a noticeable difference between the density of growth in the filtrate and control tubes seeded with *S. oranienberg*. Inhibitory action also was obtained when Ruy's medium and tetrathionate broth to which filtrate had been added were seeded with *S. choleraesuis*.

The bacteriophage was passed in series in young broth cultures of *S. choleraesuis* var. *kunzendorf*. The fourth passage inhibited growth of this organism up to the seventh 10-fold dilution. The same passage produced plaques on agar plates seeded with this organism up to the eleventh 10-fold dilution. Generally, passages were made every day but occasionally there were two or three days between passages. The eightieth passage appeared to be the same as the fourth.

The first passage of the bacteriophage was made by adding 0.5 cc. of the filtrate to a broth culture 3 or 4 hours old and incubating it at 37 C. for twenty-four hours. For subsequent passages, the following procedure was used: Two tubes of broth were seeded with the same strain of *S. choleraesuis* as used in the first passage. After three to four hours incubation, 0.5 cc. of the preceding culture, to which

From the Pathological Division, Bureau of Animal Industry, Agricultural Research Administration, USDA. (Dale and Schoening). Doctor Meriweather resigned from the Bureau Nov. 20, 1943.

bacteriophage had been added, was seeded in one of the young broth cultures. The second broth culture served as a control and was used for seeding the two tubes of broth for the following passage. The control tube and the tube containing bacteriophage were incubated until the following passage was made.

TABLE I—Results of Tests for Bacteriophage in Feces of Young Pigs

Pig No.	Age of pigs in weeks				
	3	5	7	9	11
1	—	+	—	—*	+
2	+	+	—	—	+
3	—	+	—	+	+
4	—	+	+	—	—*
5	—	—	—	—	—*
6	—	+	+	+	+
7	—	+	+	+	—*
8	—	—	+	—*	—*
9	+	+	—	—*	—*
10	—	—	+	—*	—*
11	+	+	+	+	—*
12	—*	+	—	—*	+
13	—	—*	—*	+	
14	—	+	—*	—*	
15	+	+	—*	+	
16	—	+	—*	—*	
17	—	+	+	+	
18	—	+	—*	0	
19	—	—*	—*	+	
20	—	+	—*	—*	
21	—	+	+	+	
22	—	+	+	+	
23	—	—	—*	—*	
24	—	—*	+	+	

+ = positive; — = negative; 0 = not tested; * = confirmed by testing single passage of filtrate with *S. choleraesuis* var. *kunzendorf* for concentration of bacteriophage.

To test further the inhibitory action of the bacteriophage, 1 part of concentrated bacteriophage was added to 9 parts of broth, and tubes of such medium were seeded with a 1-mm. loopful of 24-hour broth cultures of six strains of *S. choleraesuis* and 12 other types of Salmonella. Likewise, tubes of broth containing no bacteriophage were seeded as controls. Only one of the six strains of *S. choleraesuis* has been identified serologically. It was the *kunzendorf* type. The other 12 types of Salmonella were *typhimurium, senftenberg, pullorum, california, anatum, paratyphi B, derby, worthington, meleagridis, gallinarum, abortivoequina,* and *oranienberg.* This medium seeded with the six strains of *S. choleraesuis* remained clear for at least forty-eight hours, while the same kind of medium seeded with the other 12 types yielded within twenty-four hours a heavy growth of approximately the same density as the control tubes to which no bacteriophage had been added.

The presence of the bacteriophage in the feces might have been overlooked had it not been tested for its inhibitory property rather than its ability to lyse young broth cultures. The addition of the bacteriophage to young broth culture is followed by bacteriostatic and apparently bactericidal action. Multiplication continues, however, to the extent that in twenty-four hours a good growth occurs but the density is markedly less than that in controls.

Mouse Protective Test

Following the procedure used by Ward[3] in demonstrating the protective action of bacteriophage in typhoid infection in mice, concentrated bacteriophage was injected intravenously into mice at two-, four-, six-, and eight-hour intervals following intraperitoneal injections of approximately 500,000 organisms of *S. choleraesuis.* The mortality was 100 per cent in treated as well as control mice.

Feces Tested for Bacteriophage

Bacteriophage was present in the fecal samples of 40 normal pigs kept at the Animal Disease Station at Beltsville, Maryland.

Two fecal samples from a herd in Virginia were positive for bacteriophage while nine fecal samples from another herd were negative.

Fecal samples from 32 herds in Nebraska have been examined for the presence of bacteriophage. Three or four samples were obtained from each herd. All the samples from 15 herds were positive, all from 10 herds were negative, and from 7 herds, some samples were positive and some negative. At the time the samples were collected, outbreaks of disease were present in all the herds except one. Hog cholera was suspected to be associated with 19 of the outbreaks. Bacteriophage was present in the fecal samples from 10 of these 19 herds. Bacteriophage was also found in the fecal samples from 10 of the 12 herds in which some disease other than hog cholera was suspected. In one of the two herds in which the samples of feces were negative for bacteriophage, no enteritis was present but, previously, swine on the premises had been affected with enteritis for several years. While salmonellosis was suspected in the second herd in which no bacteriophage was found, improper feeds and feeding were considered possible causes of the trouble as no cases of necrotic enteritis had occurred on the place for several years.

Specimens of tissues from affected ani-

mals were examined bacteriologically in 11 of the 19 suspected hog-cholera cases. *S. choleraesuis* var. *kunzendorf* was obtained from 4. Of the other 12 outbreaks, specimens of tissues from affected animals were examined in 6 cases. *S. choleraesuis* was not obtained in any of these.

Fecal samples from young pigs weaned at 3 weeks of age and maintained on purified feeds have been examined for bacteriophage at approximately two-week intervals. The samples were obtained from a group of 12 pigs over a period of eight weeks and from a second group of 12 pigs over a period of six weeks. The results are recorded in table 1. At the time that the pigs were put on purified rations, they were isolated in individual pens with wire floors. The samples of feces were collected in sterilized containers by aseptic methods from the wire floors when available and otherwise from the rectum. In most of the cases, there were irregularities in the presence and absence of bacteriophage in the different samples from the same pig. At this time, we have no explanation to offer for these irregularities.

DISCUSSION

Bacteriophage in the feces of swine presents two problems which are quite significant. One is the extent to which its inhibitory action can affect the results of bacteriologic examinations of swine feces for the presence of Salmonella. The second is the extent, if any, to which the presence of the bacteriophage may modify the manifestations of disease that *S. choleraesuis* and other pathogenic Salmonella are capable of producing in swine.

The tests recorded in this paper show that the bacteriophage has sufficient bacteriostatic and possibly bactericidal action to prevent the usual multiplication of *S. choleraesuis* var. *kunzendorf* when the feces of swine are cultured in beef broth, tetrathionate broth, and Ruy's medium. There have been many cases of necrotic enteritis reported where bacteriologic examinations of feces have failed to reveal the presence of *S. choleraesuis*, the organism commonly associated with the etiology of the disease. Such negative results could be expected when bacteriophage is present in the feces.

Several investigators found it difficult to produce necrotic enteritis in swine consistently by feeding broth cultures of *S. choleraesuis*. Results have been more satisfactory when large doses were used. Frequently, the degree of virulence of the culture employed was thought to account for the variations in the extent of disease produced. It seems plausible that the presence or absence of bacteriophage is also a factor to be considered in interpreting the results obtained when exposing swine to *S. choleraesuis*.

SUMMARY

Tests for the presence of bacteriophage for Salmonella in the feces of swine were positive in many cases and negative in others.

The inhibitory action of bacteriophage may be responsible for negative findings in bacteriologic examinations of the feces of swine for Salmonella.

The results suggest that the extent to which *S. choleraesuis* is capable of producing disease in swine may be modified by the presence of bacteriophage.

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Salmonella Infections in Turkeys

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SALMONELLA INFECTIONS cause severe losses among turkey poults. The losses occur chiefly during the first few weeks of the poult's life. Losses due to pullorum infections from as few as 5 per cent to as many as 90 per cent have occurred in some of the turkey flocks in this state.

The rapid whole-blood method of testing for pullorum disease in chickens is used in Minnesota in connection with the control program with this disease. We firmly believe that satisfactory progress is being made in the control of pullorum disease in chickens by this method of testing. The rapid whole-blood test is not sufficiently accurate for the detection of Salmonella infections in turkeys. The test tube method of testing for pullorum disease in turkeys appears to be more reliable and satisfactory. This is unfortunate since by the rapid whole-blood technique, a program of control of this disease in turkeys could be expedited at considerably less cost.

A considerable number of turkey breeders in this state were employing the rapid whole-blood test for the detection of pullorum disease in their flocks. They were using the same antigen as was being used in connection with the testing of chickens. As a result, many of the flocks so tested were reported to be negative while others were said to have only a low percentage of reactors. However, when poults hatched from eggs produced in such flocks began to arrive in our laboratory, it was found that many harbored Salmonella organisms. The frequency of this occurrence suggested that a study be made of the two methods of testing turkeys; namely, the rapid whole-blood and the test tube methods. This soon led to a comprehensive investigation of testing turkeys for Salmonella infection.

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LITERATURE

Hewitt[1], in 1927, was the first to report the presence of pullorum disease among turkey poults. The records of the Diagnosis Laboratory at University Farm[2] show that the internal organs of 41 per cent of the lots of poults that were examined during the spring of 1942 were harboring *Salmonella pullorum*. Paratyphoid organisms were recovered from an additional 18 per cent of the lots examined. Hinshaw *et al.*[3], in comparing the results of the rapid whole-blood test, tube test, and bacteriologic examination of the internal organs of reactors, found that the rapid whole-blood test was approximately one-half as efficient as the tube test. The conclusion was made on the results of the bacteriologic studies. These authors recommended the tube test, using a 1:25 dilution, as the most desirable diagnostic method. Hinshaw and McNeil[4] reported on the progress of an eradication project of pullorum disease from turkey flocks by the use of the tube test. During the 1939-1940 season, 49,890 tests were made, which disclosed 1.77 per cent of the males and 4.23 per cent of the females as reactors. During the 1940-1941 breeding season, Weisner of Michigan found only 0.518 per cent infection in 6,953 birds,[5] using the tube test. At the veterinary diagnostic laboratory, Iowa State College, 28,147 tests were made by the tube test which disclosed 3 per cent of infection.[6]

METHODS OF PROCEDURE

During the 1941 breeding season, through the coöperation of Dr. L. E. Jenkins of the State Livestock Sanitary Board, turkeys were tested on farms by the rapid whole-blood method. At the same time, blood was collected from each of the turkeys tested and sent to the laboratory where the test tube method of testing was conducted. Turkeys were obtained from these flocks and brought to the laboratory. On the basis of the test results, these turkeys were divided into two separate groups—group 1 and group 2.

Group 1 contained 30 turkeys that reacted to one or both tests. Group 2 contained 26 turkeys whose blood serums, when tested with

rapid whole-blood plate or tube antigens, produced aggregates or clumps that we and others have described as "fine" reactions. This type of agglutination quite often causes one to pause and ponder how to interpret such phenomena. The turkeys were trap-nested and eggs collected for hatching. At the end of three weeks incubation, the eggs were candled and all those containing dead embryos and the infertile ones were removed. The poults were pedigreed and maintained in batteries for four weeks and then transferred to pens in a brooder house. The adult birds were tested at regular intervals. Comparative studies were made by using three official methods of testing recognized by the National Poultry Improvement Plan[7] in the control of pullorum disease of chickens. The procedures for culturing the eggs and bacteriologic examination of the adult turkeys and baby poults have been described.[13,14] The heart's blood, liver, spleen, ovary, lung, crop contents, contents of the duodenum, the median and posterior parts of the intestine, and the cloaca of the adult turkeys were cultured. The heart's blood, liver, yolk sac, and contents of the intestinal tract of the poults were cultured.

For the comparative studies between the rapid whole-blood and the tube methods, 2 rapid whole-blood plate antigens and 1 pullorum tube antigen were used: an experimental rapid whole-blood antigen, identified as (1), prepared by the United States Bureau of Animal Industry; a rapid whole-blood T. G. antigen,* identified as (2), prepared in our laboratory, and used as the official antigen for testing approximately one million chickens; and a test tube antigen, identified as (3), prepared by using three pullorum strains of chicken origin furnished by Dr. L. F. Rettger. The rapid whole-blood test was conducted by using 1 drop (0.04 cc.) of antigen and 1 loopful of whole blood (0.02 cc.). Three dilutions were used in the tube method (1:25, 1:50 and 1:100). One cc. of antigen was used. The samples were incubated at 37.5 C. and read at the end of forty-eight hours of incubation. Complete agglutination in the 1:25 dilution was considered a reactor. Incomplete agglutination in the same dilution was designated as a suspect.

The typhi-murium tube antigen identified as (4) was prepared from *Salmonella typhi-murium* strains isolated in our laboratory.

The serum plate test was conducted by using 0.04 cc. of serum and 1 drop of antigen, identified as (5). The test was read at the end of five minutes. The serum plate antigen (5) was a 3 per cent suspension of the same organisms used in preparing the tube antigen (3). A complete agglutination reaction was considered a reactor and an incomplete agglutination as a suspect.

Table 1 contains the data that were obtained by testing turkeys with rapid whole-blood experimental antigen (1) and with pullorum tube antigen (3).

Table 2 shows the results of testing turkeys by using T. G. antigen (2) and pullorum tube antigen (3).

In both groups of turkeys included in tables 1 and 2, there were individuals that were positive with one antigen and suspicious with the other antigen. There were 51 such turkeys in table 1 and 23 in table 2.

During the 1941 breeding season, 3 flocks were tube tested two or more times with pullorum tube antigen (3). The following are the results: The first test of flock 1 disclosed 23 reactors, 11 suspects, 207 negative; second test, 12 reactors, 196 negative; third test, 2 reactors, 181 negative; and fourth test, 3 reactors, 172 negative. Thus, by monthly retests, the initial infection was reduced from 9.5 per cent to 1.7 per cent. The initial test of flock 2, containing 1,265 turkeys, disclosed 4.8 per cent of infection. Four months later the flock was marketed and at that time 122 random blood samples were collected and tested, disclosing 20.5 per cent of infection. Flock 3 contained 427 turkeys and the initial test disclosed 12.4 per cent of infection. The flock was retested four months later and the infection was 13.1 per cent. In each instance, all reactors were removed while the flocks were in egg production.

During the 1942 breeding season, 14 flocks containing 5,900 turkeys were tube tested with pullorum (3) and typhi-murium tube (4) antigens. The pullorum antigen disclosed 6.1 per cent of reactors and 7.1 per cent suspects. Five thousand one hundred and four (5,104) turkeys were tested with typhi-murium antigen disclosing 2.5 per cent reactors and 7.4 per cent suspects. Pullorum disease within these flocks varied from a low of 0.9 per cent to a high of 30.5 per cent as determined by the tube agglutination test. Flock infection was reduced in one flock from 4.0 per cent in 1941 to 2.5 per cent in 1942, yet when this flock was marketed, a group of random samples were tested and a 10.3 per cent of infection was found. Another flock with a 9.5 per cent of infection in 1941, when retested in 1942, a 0.2 per cent infection was found, yet when the flock was marketed an 8.1 per cent infection was disclosed. Other data not included in this study compare with the above; that is, in our experience infection was found higher at marketing time than when tested before egg production began.

*The production of pullorum-disease stained antigen from a Thiosulphate-Glycerin culture medium. (Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture, Feb. 28, 1940).

Considerable variation in the amount of infection was found when flocks were tested by the rapid whole-blood and test tube methods. The former disclosed an average of 1.8 per cent of infection while the latter was higher—6.6 per cent. Many of the reactions with rapid whole-blood antigens that were used were difficult to interpret; that is, in too many instances, the agglutinations that occurred were not definite—difficult to satisfy one's self as to whether the turkey was a reactor or not, since the reaction that occurred was definitely not negative. This factor probably accounts for some of the variation in results reported.

TABLE 1

Number of flocks tested	26
Number of turkeys tested	4,954
Number of reactors with experimental antigen (1)	128*
Number of reactors with test-tube antigen (3)	212†
Number of reactors with both antigens (1, 3)	71
Number of suspects with experimental antigen (1)	30*
Number of suspects with test-tube antigen (3)	78*

*These turkeys were negative with tube test antigen (3).

†These turkeys were negative with the rapid whole-blood experimental antigen (1).

STUDY OF THE TURKEYS IN GROUP 1

These are the 30 birds mentioned earlier and were brought to the laboratory for further study. The first time they were tested they were classified as follows: by the rapid whole-blood test, 7 reactors, 5 suspects, 14 negative, and 4 were not tested; with pullorum tube antigen (3), 27 reactors, 2 suspects, and 1 negative; with typhimurium tube antigen (4), 15 reactors and 15 negative; serum plate test antigen (5), 9 reactors, 1 suspect, 1 negative, and 19 were not tested. Cross agglutination occurred in approximately 50 per cent of the turkeys. The turkeys were retested six times at intervals of approximately six weeks. Sixteen turkeys either died or were destroyed because of severe illness before the end of the period of observation. The mortality was attributed mainly to an air sac infection. Just previous to the time the birds were destroyed, the tube test (3) revealed 12 negative, 13 reactors, and 5 suspects. The serum plate test (5) showed 8 negative, 11 reactors, and 11 suspects. The rapid whole-blood test (1) disclosed 15 negative, 9 reactors, and 6 suspects. It was demonstrated that not all reactors continue as such regardless of the method of test used.

Reactors may become suspects or even negative; suspects may and do become reactors, some continue as suspects or become negative; and some negative turkeys, when in the same pen with reactor turkeys, become suspects or reactors. This happened with the tom that was negative at the beginning of the experiment but became a reactor during the fourth retest. Three other toms in the same pen remained negative. The tube test consistently disclosed more reactors than did the rapid whole-blood test. In summarizing, it may be stated that of the original 27 reactors, as disclosed by the pullorum test tube antigen (3), 17 remained consistently reactors during the period of study. Nine gradually lost or showed lower titers than the initial test, and 3 consistently reacted until the last retest when they became negative.

S. pullorum was recovered from 13 of the 27 turkeys that were originally designated as reactors by the tube test, and *Salmonella newport* from 3 other turkeys. One turkey that was a reactor on the last test died, and *S. pullorum* was not isolated from the internal organs or eggs laid by this turkey; but *Pseudomonas aërogenes* was isolated from all of the internal organs. From the tom that was negative on the first test and became a reactor on the last test, *S. pullorum* was recovered from the liver and spleen. The 2 suspects on the original tube test were negative on the last test and Sal-

TABLE 2

Number of flocks tested	21
Number of turkeys tested	2,679
Number of reactors with T. G. antigen (2)	68*
Number of reactors with test-tube antigen (3)	91†
Number of reactors with both antigens (2, 3)	27
Number of suspects with T. G. antigen (2)	7*
Number of suspects with test-tube antigen (3)	23†

*These turkeys were negative with tube test antigen (3).

†These turkeys were negative with the rapid whole-blood experimental antigen (2).

monella was not isolated from the eggs laid or from the internal organs of these hens. The final results of the bacteriologic examination of the internal organs of the turkeys in Group 1 were as follows: *S. pullorum* from 14, *S. newport* from 3, Proteus organisms from 6, indol positive organisms from 2, a paracolon organism from 1, a Pseudomonas organism from 3, and 1 turkey was negative.

TABLE 3

Turkey	Eggs Produced	No. Infected	Turkey	Eggs Produced	No. Infected
1	8	3	7	31	1
2	21	2	8	17	1
3	7	4	9	24	1
4	19	3	10	26	1
5	8	3	11	3	1
6	1	1	Floor Eggs*	81	2

*Eggs found on the floor and could not be identified.

The turkeys produced 507 eggs during the laying period. There were 221 poults hatched and 286 eggs failed to hatch. The latter was comprised of 187 infertile eggs, 24 embryos that were dead when candled at 21 days and 75 embryos that failed to hatch. *S. pullorum* was recovered from 7.3 per cent of the infertile eggs and 11.1 per cent of the embryos.

S. pullorum was isolated from eggs laid by 11 turkeys. Table 3 shows the number of eggs produced by these hens and the number of eggs that were found infected with *S. pullorum*.

S. pullorum was recovered from the eggs of 5 turkeys but was not recovered from the internal organs of the turkeys that produced the eggs. From another turkey, *S. newport* was recovered from the intestinal tract, but *S. pullorum* was recovered from 3 of 8 eggs laid by this hen. *S. pullorum* was isolated from eggs and internal organs of 4 turkeys, but in contrast to this experience we isolated the organism from the internal organs of 4 other turkeys but not from their eggs. Eggs were not recorded as laid by 4 turkeys yet *S. pullorum* was recovered from their internal organs; they either contributed floor eggs or were nonproductive. *S. newport* was recovered from 2 turkeys but their eggs were sterile.

The 221 poults were started in batteries for the first four weeks. Each hatch was kept as a unit until 6 weeks of age, at which time they were placed in a brooder house.

TABLE 4

Turkey	Test I				Test II				Test III				Test IV				Test V				Test VI				Results of Bact. Exam. of Organs	Results of Bact. Exam. of Eggs
	TP	TT	WB	SP	TP	TT	WB	SP	TP	TT	WB	SP	TP	TT	WB	SP	TP	TT	WB	SP	TP	TT	WB	SP		
1	+	—	s	s	s	—	s	s	s	—	s	s	—	—	s	s	—	—	—	—	—	—	—	—	P 5	+
2	+	—	—	+	+	—	+	+	+	s	s	+	+	—	s	+	s	—	s	s	—	—	—	—	Sp 1, 4	+
3	+	+	—	+	s	—	s	s	+	s	s	s	—	—	—	—	D								Ps 4, 5	—
4	+	s	+	+	—	+	s	s	—	—	s	s	—	—	—	—	—	—	—	s	—	—	—	—	I 5	—
5	+	—	o	+	—	—	—	s	—	—	—	—	s	—	—	s	—	—	—	—	K				Ps 3	+
6	+	+	—	o	—	—	s	s	—	—	—	s	—	—	s	s	s	s	s	s	—	—	—	—	Negative	+
7	+	+	o	o	s	+	s	+	+	+	+	+	s	—	s	s	s	s	s	s	s	s	s	s	Sn 1, 2	—
8	+	+	—	o	s	+	s	+	+	—	—	s	s	—	s	s	D								Sp 1, 2, 3, 4, 5	—
9	+	+	—	o	+	s	+	+	+	—	+	+	+	+	+	+	+	s	+	+	+	s	+	+	Sp 1, 2, 3, 4	+
10	+	+	s	o	+	—	+	+	D																Ps 1, 2, 3, 4, 5	—

Legend for agglutination tests: TP = Tube pullorum; TT = Tube typhi-murium; WB = Whole-blood pullorum test; SP = Serum plate test; + = Reactor; — = Negative; s = Suspect; D = Died; K = Killed; o = Not tested.

Legend for organisms isolated and the organs harboring the same: Proteus = P; *S. pullorum* = Sp; *S. newport* = Sn; Indol positive organism = I; Pseudomonas = Ps; Paracolon = Pa; 1 = Liver; 2 = Spleen; 3 = Lung; 4 = Ovary; 5 = Intestinal Tract.

Legend for eggs examined: + = *S. pullorum* isolated; — = Negative; o = No eggs examined.

One hundred and twenty-three (123) poults died or were destroyed and were examined bacteriologically. *S. pullorum* was recovered from 8 poults that died during the first four weeks. *S. newport* was recovered from 1 poult but none of the poults produced by the 3 adult turkeys found harboring *S. newport* was infected with this organism. Seven of the 8 infected poults belonged to the same hatch and were from adults from which *S. pullorum* was isolated. The remaining infected poult was hatched at an earlier date and no other poults in that hatch died from pullorum disease. The hen that produced this poult and her eggs that were examined were found negative.

The majority of the 30 turkeys in group 1 were retested five and six times. Table 4 includes 10 turkeys that are considered

TABLE 5

	NUMBER TESTED	NUMBER OF REACTORS	NUMBER OF SUSPECTS
1st test	138	5	5
2nd test	103	3	5
3rd test	136	3	2
4th test	82	1	7

representative individuals and shows the data obtained. The rapid whole-blood tests (1) and the rapid serum plate tests (5) were made by using different experimental stained antigens that were supplied to us or were similar products prepared in our laboratory. The results of the bacteriologic examination of the internal organs and the eggs that failed to hatch are included.

STUDY OF GROUP 2

This group contained 26 turkeys whose blood serums, when tested with rapid whole-blood plate or tube antigens, produced aggregates or clumps that we and others have called "fine" reactors. They had been tested and retested during their growing period and at one time or another were found to have had an agglutination titer at 1:25 dilution, but never higher than 1:100 dilution. They were also tested repeatedly during the laying period of five months. The maximum titer of some of the turkeys was never higher than the 1:25 dilution. At one or more times, 19 of the turkeys were negative. Seven of the turkeys constantly carried a titer ranging from 1:25 to 1:100 dilution. The antigens and methods of testing were the same as used in group 1. At the end of the laying period, all of the birds were examined bacteriologically. *S. pullorum* was not recovered from any of the turkeys. *S. newport* was recovered from the intestine of 1 and from the cloaca of a second turkey. Organisms other than Salmonella recovered were as follows: Proteus from 10 turkeys, Proteus and paracolon from 1 turkey, indol positive Salmonella-like organisms from 1 turkey, and paracolon from 3 turkeys.

Seven hundred and forty-five (745) eggs were collected and 234 poults were hatched. There were 371 infertile eggs, 16 embryos dead at 7 days, 15 embryos dead at the fourteenth day, and 109 poults failed to emerge from the shell at the end of the incubation period. *S. pullorum* was recovered from 2 eggs containing full term embryos and from 4 infertile eggs. These eggs were produced by 6 different hens. The 133 poults that died were examined bacteriologically and Salmonella organisms were not recovered.

STUDY OF THE POULTS FROM GROUPS 1 AND 2

The poults were tested repeatedly beginning at 8 to 10 weeks of age and continued until they were marketed or were selected for breeders. They were tested by the rapid whole-blood method and the tube method using pullorum and typhi-murium antigens. The rapid whole-blood plate antigens disclosed very few reactors throughout all tests. Cross agglutination with pullorum and typhi-murium tube antigen was inconsistent. Table 5 gives the results of testing these turkeys with pullorum tube antigen.

Two of the turkeys that were negative on the first test were suspects on the second test, 3 suspects continued as such, 1 bird continued as a reactor, the other 2 reactors were negative to the first test. Third test—1 turkey continued as a reactor, the other 2 reactors were negative to the second test. At the completion of the

third test, some of the negative turkeys were marketed. The 1 reactor found on the fourth test had been negative to all previous tests and 1 turkey that had reacted on the previous test became a suspect. The remaining 6 suspects had been negative to all previous tests.

It appears that many young poults that may survive an original exposure and yet show evidence of infection as indicated by the agglutination test gradually lose the infection and become negative by the time they reach breeding age. A small percentage may retain the infection and act as carriers. Two pens of breeders were selected from the flock of 82 turkeys with which to continue our studies.

DISCUSSION

The control of pullorum disease in adult turkeys in Minnesota has been attempted by the use of the rapid whole-blood method. Comparative studies of the whole-blood method and tube test were made on 4,954 turkeys. Fifty-eight per cent of the turkeys designated reactors by the rapid whole-blood plate method were negative by the tube test and 70 per cent of the reactors as shown by the tube test were negative by the rapid whole-blood plate method. The results of the comparative studies of the two other official methods of testing, as applied to turkeys constituting group 1, revealed differences but not as marked. The tube test and the rapid serum plate methods agreed quite well, but the rapid whole-blood plate method was far from being in agreement.

In this study, as well as in previous publications relating to the adult carrier problem, organisms belonging to the Enterobactereaceae were isolated.[13,14] The majority of the proteus strains were identified as *Proteus mirabilis*. The organisms designated as paracolons and indol positive Salmonella-like organisms could not be satisfactorily identified. Sanders, Pomeroy, and Fenstermacher[8] found that these organisms produced agglutinins that would cross agglutinate with the pullorum tube and serum plate antigens in low dilutions. Bunyea and MacDonald[9] showed that some strains of *Aërobactor aërogenes* and *Escherichia acidi lactici* of turkey origin possessed cross-agglutinating ability in the presence of pullorum antiserum and vice versa. Schiff, Bornstein, and Saphra[10] described a culture of *Escherichia coli* which contained antigens of the Salmonella. Peluffo, Edwards, and Bruner[11] reported that the 7 paracolon strains studied possessed flagellar antigens closely related to those of *Salmonella dussendorf* and *Salmonella cerro*. Johnson and Pollard[12] have observed that turkeys infected with a gram-positive organism belonging to the Lactobacilli group may cause non-specific reactions in 1:25 dilution. These cross reactions with pullorum antigens may be one of the explanations why some birds removed as reactors on one test may on subsequent tests be negative.

It has been the customary practice in Minnesota to confine the breeding stock in yards close to the farm buildings or in complete confinement in permanent laying houses. The constant occurrence of reactors in flocks that have been retested several times may be partly attributed to pen contact and the eating of infected eggs. Some of the yards have been used for several seasons and infection may be derived from the contaminated soil. It is essential that certain management, particularly sanitary practices, be carried out along with testing programs.

SUMMARY

1) We have isolated from poults, 23 types of organisms belonging to Salmonella. One additional type, *Salmonella rubislaw*, was identified by Edwards and Bruner. They typed serologically the strains of Salmonella isolated during 1941. *Salmonella pullorum* was most frequently encountered in a majority of the outbreaks of salmonellosis. *Salmonella typhi-murium* was found in approximately 75 per cent of the outbreaks that were designated as paratyphoid.

2) *S. pullorum* was recovered from the internal organs of 14 and *Salmonella new-*

port from 3 of the 30 turkeys in group 1. *S. pullorum* was isolated from eggs laid by 11 of the birds in this group. 8.04 per cent of the eggs produced by group 1 were found infected with *S. pullorum*.

3) The serums of the 26 turkeys in group 2 gave "fine" aggregates or clumps when tested with tube antigens. *S. pullorum* was recovered from 0.8 per cent of eggs examined. *S. newport* was recovered from 2 turkeys and *S. pullorum* from none.

4) Organisms belonging to the Enterobactereaceae were isolated from the internal organs of several of the turkeys in group 2. These organisms and other closely related bacteria may possess antigens closely related to those of Salmonella and the presence of these infections might tend to complicate the testing of turkeys for pullorum disease.

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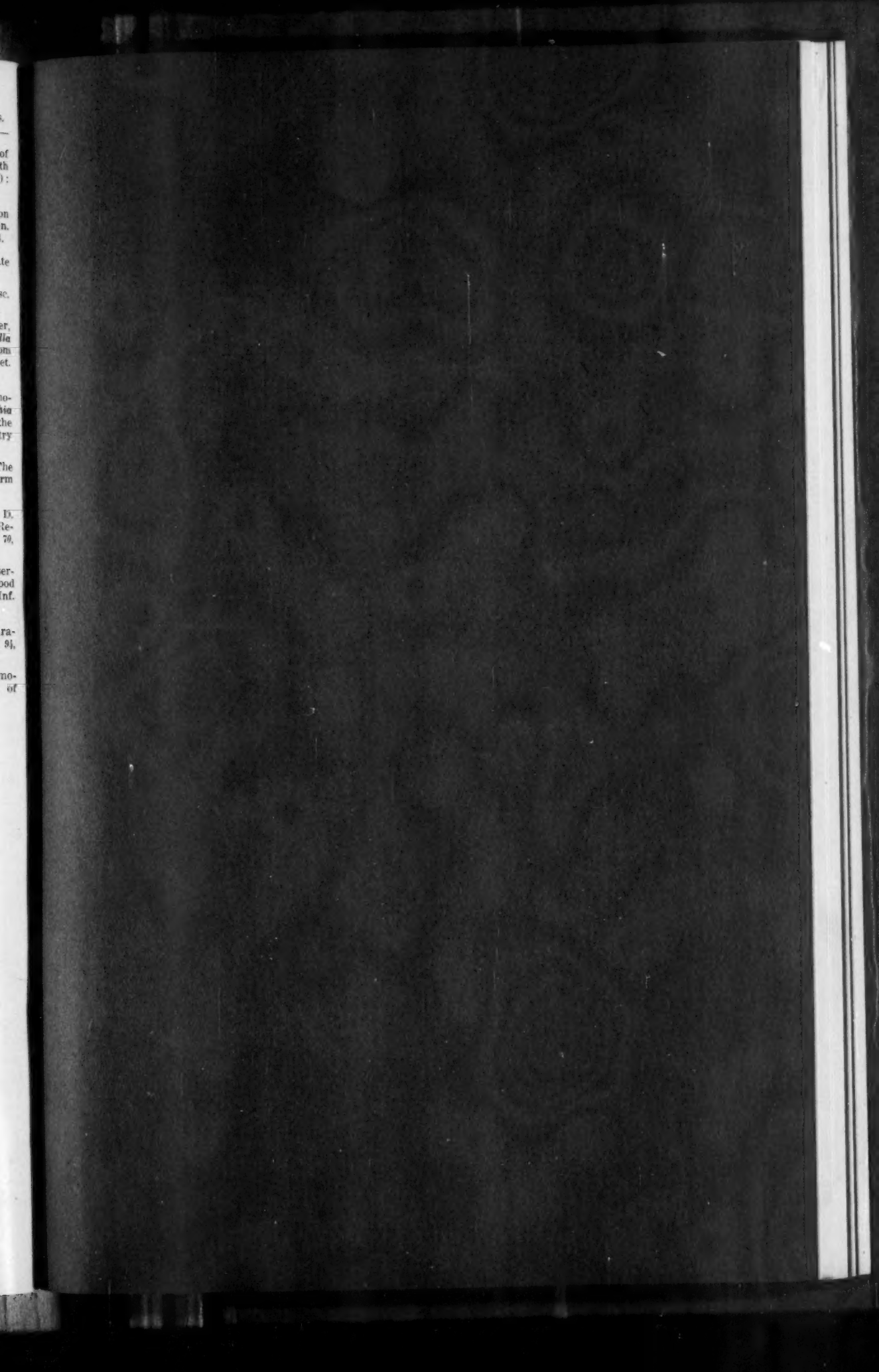
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